DETONATION NANODIAMOND PARTICLES MODIFIED BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS *IN VITRO* EXAMINATION

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Abstract

Most recently it has been found that nanodiamond particles have very interesting properties. There are number of research communications that detonation nanodiamond particles (NDPs) are fairly reactive and their surface can be effectively modified by chemical methods. The hydroxyl-modified NDPs were obtained by Fenton reaction, amine-functionalized NDPs were obtained by chemical reduction of the nitro-functionalized surface and carboxyl-modified NDPs by oxidation by using H_2O_2 under acidic conditions. NDPs functionalized by hydroxyl- and amine- groups and amino groups were used for covalent binding of non-steroidal anti-inflammatory pharmaceuticals (aspirin, ketoprofen, ibuprofen, naproxen) via ester or amide bonds. These results of the studies proved the activity of the conjugates of active substance-NDP and study the rate of release of active substance from the NDPs surface by in vitro examinations with mouse fibroblasts.

The progress of the reaction and the characteristics of the products were determined by using FT-IR. Chemical and physical structures of materials were also investigated by Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). DRIFT spectra show the modification of nanodiamond by ketoprofen, naproxen, ibuprofen and aspirin.

Keywords: *detonation nanodiamond particles, chemical modification, anti-inflammatory drugs, FT-IR spectroscopy, mouse fibroblasts*

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Introduction

Recent studies on application of carbon nanomaterials for biological purposes revealed that nanodiamonds are much more biocompatible than other carbon nanomaterials. The non-cytotoxic properties of nanodiamond particles together with other properties make them attractive for various biomedical applications both *in vitro* and *in vivo* [1]. Nanodiamond particles possess a unique set of properties attractive for drug delivery applications, including exceptional biocompatibility, large carrier capacity and versatile surface chemistry properties, which enhance drug binding and provide sustainable drug release.

Nanodiamond particles were obtained using Danilenko detonation method (discovered in 1963 in Soviet Union [2]) from graphite in presence oxygen-deficient TNT (2-methyl-1,3,5-trinitrobenzene)/hexogen composition in inert media. Detonation nanodiamond consists of nanodiamond particles (grain size from 2 to 10 nm), but the particles tend to aggregate [2-4]. Detonation nanodiamond particles are hydrophilic and nanodiamond surface has the many dangling bonds, which are chemically reactive and ready for chemical functionalization [5-9].

Detonation nanodiamond particles obtained by detonation method are described in the literature as the so-called “onion-like carbon” (OLC) [10-12]. Due to the nanoscale size of e.g. detonation nanodiamond particles, such material can be used for biological studies [13,14]. The series of research began by checking the biological properties of nanodiamond particles, which differ in content of a diamond phase and a grain size [15].

Nanodiamond particles modified by Fenton reaction exhibit the phenomenon of fluorescence in the presence of a strain of *Pseudomonas aeruginosa* ATCC 9027 that can be used in microbiological diagnostics packages containing diamond nanoparticles [6].

Detonation nanodiamond particles functionalized with hydroxyl groups were verified in studies evaluating their antioxidant potential. Detonation nanodiamond particles delayed soybean oil from going rancid, which is important in the potential for using them in bioactive packaging extending the period of food consumption [7].

Functionalization of pristine nanodiamond (ND) has influenced on fragmentation of nanoparticles, wettability and intensify of catalytic properties. Oxidation of the particle surface results in the possibility of selective attachment of functional groups and molecules. The internal carboxylic groups were observed on the nanodiamond surface. Thanks to their presence, further modifications can be made through the amide linkage. In this way the surface is modified with attached amines. Chemical modification of nanodiamond by amide functionalization increased hydrophilicity of the surface. Hydrophilicity (measured by dispersibility test in water and alcohol) makes nanodiamond surface susceptible to chemical modifications which can be controlled and selected for their medical applications [16].

Modern methods of tissue engineering utilize the acellular scaffolds ex vivo or in vivo seeded with the cells. Such scaffolds must be biocompatible, athrombogenic and mechanically stable. Often the factors used for the scaffold decellularization may affect the properties of the scaffolds cause the morphological and biomechanical changes within the extracellular matrix [17-19]. Therefore, the methods which improve the biocompatibility and mechanical properties of the materials used for bioprostheses preparation are sought. For this purpose, nanodiamonds that show promising biological properties may be used [20-24]. The aim of the study was to assess the cytotoxicity of the nanodiamonds conjugated with the anti-inflammatory factors.

Materials and Methods

Methodology of chemical modification of detonation nanodiamond particles

Synthesis of nitro-modified NDP 2

Purified in ethanol nanodiamond powder (1.1 g) was placed into round bottom flask and 22 ml of 90% HNO_3 was added. The suspension was sonicated in a series of 20 x 5 min and intensively mixed on a magnetic stirrer for 72 h at room temperature. The precipitate was filtered off and washed with water until neutral pH. Modified NO_2-NDP was dried in a vacuum desiccator to constant weight and finally 1.07 g modified NO_2-NDPs **2** was obtained.

Synthesis of amino-modified NDP 3

Nitro-modified NDP (NO_2-NDP, 1 g) was thoroughly triturated in a mortar with $FeSO_4$ (2.4 g). The powder was placed into round bottom flask and 100 ml of a mixture of ethane-water (1:1) was added. Vigorously stirred suspension was refluxed for 30 min. After cooling to room temperature, a further portion of $FeSO_4$ (5 g) and ethanol (10 ml) was added to suspension. Vigorously stirred suspension was refluxed for 1 h. After cooling to room temperature, solution of ammonia (80 ml) was added to suspension. Vigorously stirred suspension was refluxed for 5 h. In the next step $FeSO_4$ (5 g) was added to the suspension and it was refluxed for 1 h. After this time, solution of ammonia (80 ml) was added once again to the suspension and vigorously stirred; suspension was refluxed for 3 h. The suspension was diluted with water (100 ml) and the precipitate was filtered under reduced pressure. The precipitate was washed with water (25 ml), 5% H_2SO_4 (25 ml), water (50 ml) and 10% NaOH (25 ml). The final product was dried in a vacuum desiccator to a constant weight.

Synthesis of triazine esters of carboxylic acids 6a-d. General procedure

Carboxylic acid (1 mmol) and DIPEA (88 μL, 0.5 mmol) were added at 0°C to a vigorously stirred solution of DMT/NMM/$TosO^-$ **5** (0.413 g, 1 mmol) in CH_2Cl_2 (5 mL). Stirring was continued until the disappearance of condensing reagent **5** (TLC analysis, staining with 0.5% solution of NBP), after the time the mixture was diluted with DMF (5 mL) and used without any isolation and purification in next steps of H_2N-NDP **3** functionalizations.

Synthesis of triazine active ester 6a derived from Aspirin 4a

Synthesis was carried out according to the general procedure. Starting materials: Aspirin **4a** (0.180 g, 1 mmol), DMT/NMM/$TosO^-$ **5** (0.413 g, 1 mmol), DIPEA (88 μL, 0.5 mmol), CH_2Cl_2 (5 mL) and DMF (5 mL).

Synthesis of triazine active ester 6b derived from Ketoprofen 4b

Synthesis was carried out according to the general procedure. Starting materials: Ketoprofen **4b** (0.254 g, 1 mmol), DMT/NMM/$TosO^-$ **5** (0.413 g, 1 mmol), DIPEA (88 μL, 0.5 mmol), CH_2Cl_2 (5 mL) and DMF (5 mL).

Synthesis of triazine active ester 6c derived from Ibuprofen 4c

Synthesis was carried out according to the general procedure. Starting materials: Ibuprofen **4c** (0.206 g, 1 mmol), DMT/NMM/$TosO^-$ **5** (0.413 g, 1 mmol), DIPEA (88 μL, 0.5 mmol), CH_2Cl_2 (5 mL) and DMF (5 mL).

Synthesis of triazine active ester 6d derived from Naproxen 4d

Synthesis was carried out according to the general procedure. Starting materials: Naproxen **4d** (0.206 g, 1 mmol), DMT/NMM/$TosO^-$ **5** (0.413 g, 1 mmol), DIPEA (88 μL, 0.5 mmol), CH_2Cl_2 (5 mL) and DMF (5 mL).

Synthesis of NDPs 7a-d modified with carboxylic acid derivatives. General procedure

To a vigorously stirred and cooled in ice-water bath (0°C) suspension of H_2N-NDP **3** (100 mg) in CH_2Cl_2 (2 mL) was added a solution of the triazine ester **6** (1 mmol) and NMM (110 μL, 1 mmol). Stirring was continued for 12 h at room temperature. The precipitate was filtered off under reduced pressure and thoroughly washed with DMF (10 mL), CH_2Cl_2 (10 mL), water (10 mL), DMF (10 mL) and CH_2Cl_2 (10 mL). The residue was dried to constant weight in a vacuum desiccator.

Synthesis of NDP 8 modified with β-alanine residue. General procedure

To a vigorously stirred and cooled in ice-water bath (0°C) suspension of H_2N-NDP **3** (500 mg) in CH_2Cl_2 (10 mL) was added a solution of DMT/NMM/$TosO^-$ **5** (2.65 g, 5 mmol), Fmoc-β-Ala-OH (1.557 g, 5 mmol) and NMM (660 μL, 6 mmol) in mixture of DMF and CH_2Cl_2 (1:1) (15 mL). The experiment was continued for 12 h at room temperature. The precipitate was filtered off under reduced pressure and thoroughly washed with DMF (20 mL), CH_2Cl_2 (20 mL), water (20 mL), DMF (10 mL) and CH_2Cl_2 (20 mL). The residue was dried to a constant weight in a vacuum desiccator. The suspension of Fmoc-β-Ala-NDP in DMF (5 ml) was added 25% solution of piperidine in DMF. The suspension was sonicated for 5 min and then vigorously stirred for 15 min on a magnetic stirrer. The suspension was centrifuged (15 min, 4000 rpm), the supernatant was decanted. To the precipitate 25% solution of piperidine in DMF was added again and all procedure was repeated three times. The precipitate was filtered off under reduced pressure and thoroughly washed with DMF (20 mL), CH_2Cl_2 (20 mL), water (20 mL), DMF (10 mL) and CH_2Cl_2 (20 mL). The residue was dried to constant weight in a vacuum desiccator.

Synthesis of NDPs 9a-d modified with carboxylic acid derivatives. General procedure

To a vigorously stirred and cooled in ice-water bath (0°C) suspension of H_2N-β-Ala-NDP **8** (100 mg) in CH_2Cl_2 (2 mL) a solution of the triazine ester **6** (1 mmol) and NMM (110 μL, 1 mmol) was added. Stirring was continued for 12 h at room temperature. The precipitate was filtered off under reduced pressure and thoroughly washed with DMF (10 mL), CH_2Cl_2 (10 mL), water (10 mL), DMF (10 mL) and CH_2Cl_2 (10 mL). The residue was dried to constant weight in a vacuum desiccator.

FT-IR spectroscopy examination

The progress of the reaction and the characteristics of the products were determined by using FT-IR. All measurements were carried out at room temperature and in air atmosphere. Chemical and physical structures of materials were also investigated by Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) using the Thermo Scientific Nicolet iS50 FT-IR spectroscope. DRIFT spectra were collected in the range of 400-4000 cm^{-1}.

Methodology of in vitro examination

To prevent cell culture contamination the diamond powders particles were exposed to solution containing antibiotics (100 U/ml Penicilin/Streptomycin, 5 mg/ml Ciprofloxacinum and 2 mg/ml Fluconazolum) at 4°C for 24 h. Mouse fibroblasts L929 (ATCC CCL-1) were used for Cytotoxicity test. Cell cultivation was performed in medium 199 with 10% fetal bovine serum, 100 U/ml Penicilin/Streptomycin, 5 mg/ml Ciprofloxacinum and 2 mg/ml Fluconazolum. The cells were growing at 37°C and 5% CO_2. When fibroblasts reached 80% confluency they were treated with the diamond powder suspensions (50 µg/ml). Cell viability was assessed after 24 h using fluorescent microscope (Zeiss AxioObserver.Z1 fluorescence microscope, 10x). For that purpose fluorescent staining were used: fluorescein diacetate (FDA, 1 mg/ml) which penetrates trough living cells membrane gives green fluorescence and propidium iodide which gives bright red light on apoptotic and necrotic cells. TABLE 1 showed methodological assumptions for the *in vitro* experiment.

Results and Discussion

One of the most popular methods of functionalization of nanodiamond particles is the incorporation of hydroxyl groups on the surface of the nanoparticles under the Fenton reaction conditions [25]. The presence of hydroxyl groups on the surface of the NDPs enables their widespread adaptation in subsequent functionalization steps by using variety of molecules. The hydroxyl-modified nanodiamond particles were obtained by Fenton reaction, amine-functionalized NDPs by chemical reduction of the nitro-functionalized surface, carboxyl-modified NDPs by oxidation with the use of H_2O_2 under acidic conditions [26].

However, direct transformation of hydroxyl groups with a carboxylic acid derivatives leads to the formation of the corresponding esters which under physiological conditions have a moderate stability. In order to eliminate the inconvenience of inadequate stability of NDPs modified with biologically active carboxylic acids (nonsteroidal anti-inflammatory drugs, NSAIDs), it has been assumed that the more stable are the corresponding amide derivatives.

Functionalization of NDPs surface by amino groups has been achieved in a two-steps reaction. The first reaction step involved the nitration of the NDPs surface under standard nitration conditions. In the second step the nitro groups were reduced to the corresponding amines [27] (FIG. 1).

The next step functionalization H_2N-NDPS (**3**) consisted of the acylation of the amino functions on the surface of NDPs by super-active triazine esters **6** of NSAIDs (aspirin **4a**, ketoprofen **4b**, ibuprofen **4c** and naproxen **4d**) with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium toluene-4-sulfonate (DMT/NMM/$TosO^-$ **5**) [27] (FIG. 2).

TABLE 1. The qualitative and morphological classification of cellular cytotoxicity.

Cytotoxicity	*Reactivity*	*Cell condition*
0	lack	Discrete intra-plasmatic granules, no lysis, no reduction of cell growth
1	slight	Not more than 20% of round cells, loosely adherent without intra-plasmatic granules, showing morphological changes, slight cell lysis, a slight inhibition of cell growth
2	mild	Not more than 50% of round cells, without intra-plasmatic granules, strong cell lysis, not more than 50% inhibition of cell growth
3	moderate	Not more than 70% a surface comprising a round and lysed cell, not completely damaged, cell growth inhibition more than 50%
4	strong	Almost total and complete destruction of cells

The choice of a triazine coupling reagents to modify the surface of NDPs was dictated by the fact that the acylation of nucleophiles with superactive triazine esters is very effective and application of them ensuring the removal of side-products (elimination of deposites on the surface of a solid matrix). 2-Hydroxy-4,4-dimethoxy01,3,5-triazine, the side product of acylation with triazine based coupling reagents is removed by extraction with polar organic solvents or by washing with water.

In order to improve the exposition of NSAIDs on the surface of the nanoparticles modified NDPs containing the rest of β-alanine as a linker between the nanoparticles and attached NSAIDs were obtained (FIG. 3).

For the acylation of amino groups of NDPs **3** DMT/NMM/$TosO^-$ **5** as a coupling reagent was used. The coupling and subsequent removal of the Fmoc group has been done by using standard synthetic protocols for solid phase peptide synthesis [28]. The stage of incorporation of acid derivatives (NSAIDs) was implemented by using appropriate triazine esters **6a-d** derived from aspirin **4a**, ketoprofen **4b**, ibuprofen **4c** and naproxen **4d**.

FIGs 4-6 show the results of FT-IR spectroscopy of nanodiamond particles without chemical modification (FIG. 4) and the attached anti-inflammatory drugs (FIGs 5 and 6). The results of FT-IR confirm the presence of amide bonds (FIG. 5) and amide bonds by a β-alanine connector.

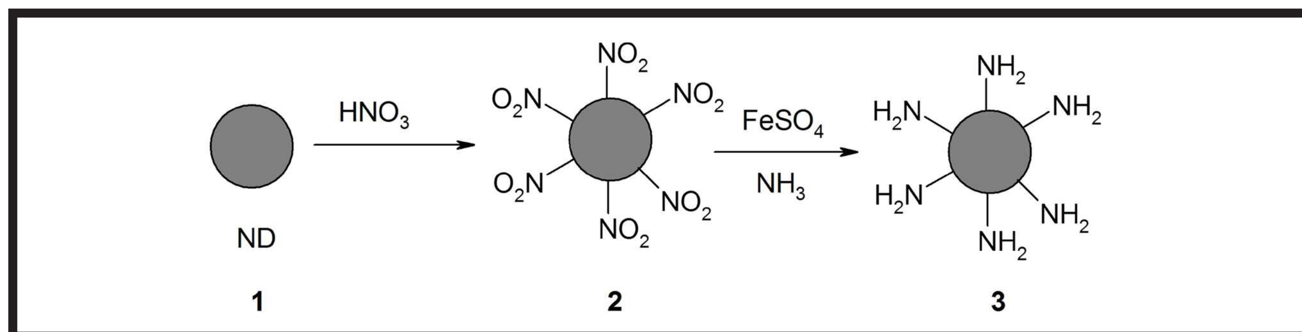

FIG. 1. Synthesis of amino-functionalized NDPs.

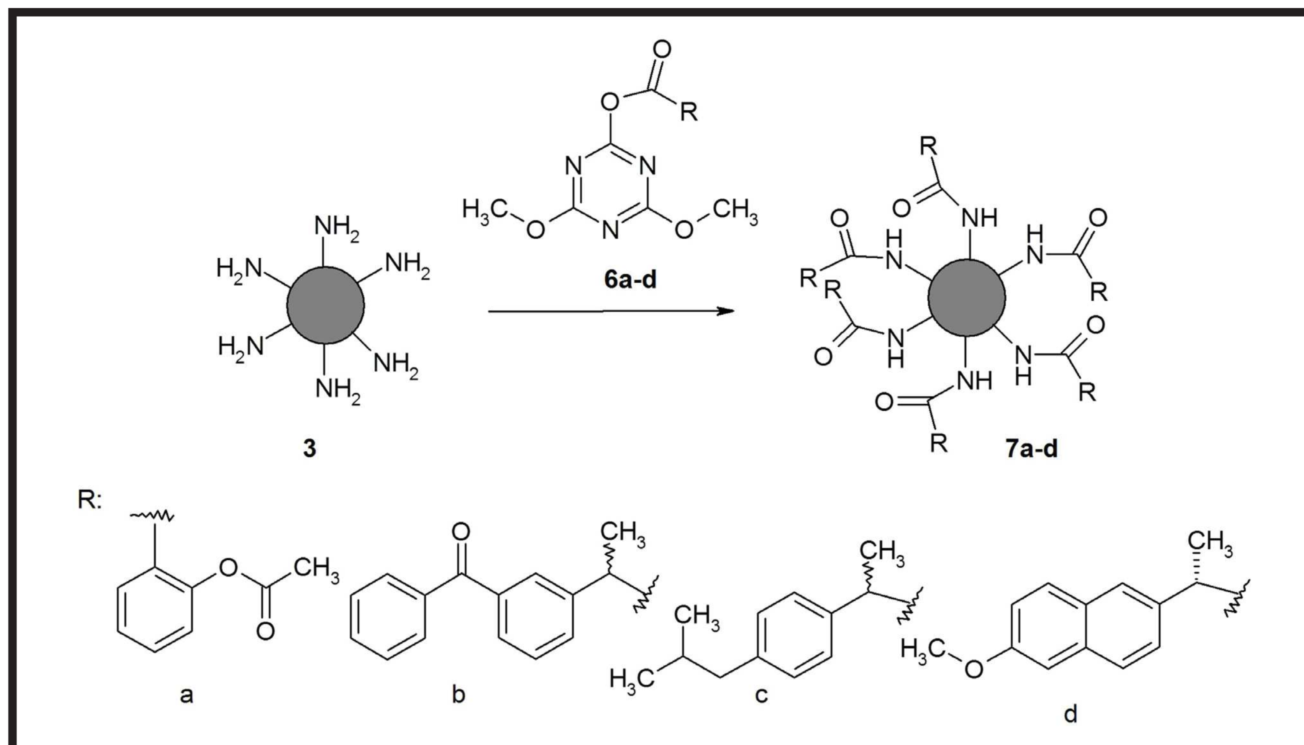

FIG. 2. Synthesis of NDPs 7a-d modified with nonsteroidal anti-inflammatory drugs.

1) Fmoc-β-Ala-OH, DMT/NMM/TOS⁻, NMM
2) 25% piperidine, DMF
3
8
6a-d
9a-d

FIG. 3. Synthesis of of NDPs 9a-d modified with nonsteroidal anti-inflammatory drugs separated from surface of nanoparticle by β-alanine residue.

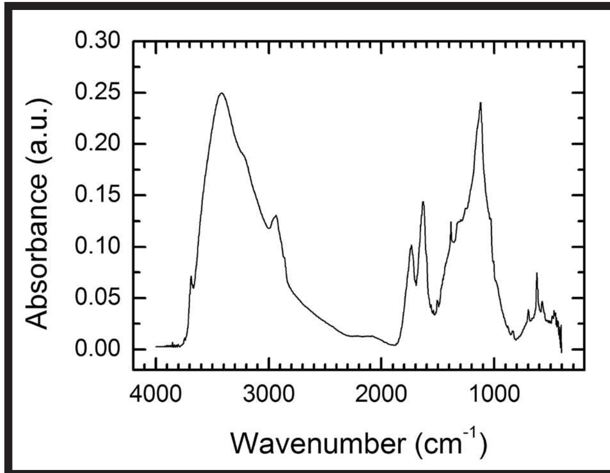

FIG. 4. FT-IR spectra of detonation nanodiamond particles chemical modification.

TABLE 2. Classification of cytotoxicity of tested materials.

Tested samples	Cyto-toxicity	Reacti-vity
NDP-NHCO-ketoprofen	1	slight
NDP-NHCO-naproksen	1	slight
NDP-NHCO-ibuprofen	1	slight
NDP-NHCO-aspirin	1	slight
NDP-NHCO-β-Ala-NHCO-ketoprofen	1	slight
NDP-NHCO-β-Ala-NHCO-naproksen	1	slight
NDP-NHCO-β-Ala-NHCO-ibuprofen	1	slight
NDP-NHCO-β-Ala-NHCO-aspirin	1	slight

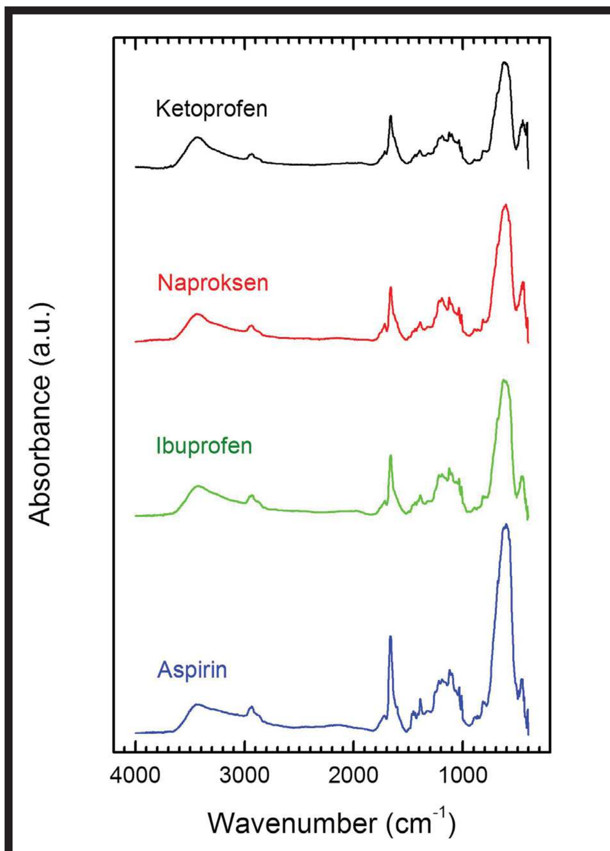

FIG. 5. FT-IR spectra of NDP with attached drugs (R-ketoprofen, R-naproksen, R-ibuprofen, R-aspirin) by ester and amine bonds and carboxyl bonds –NHCO-R .

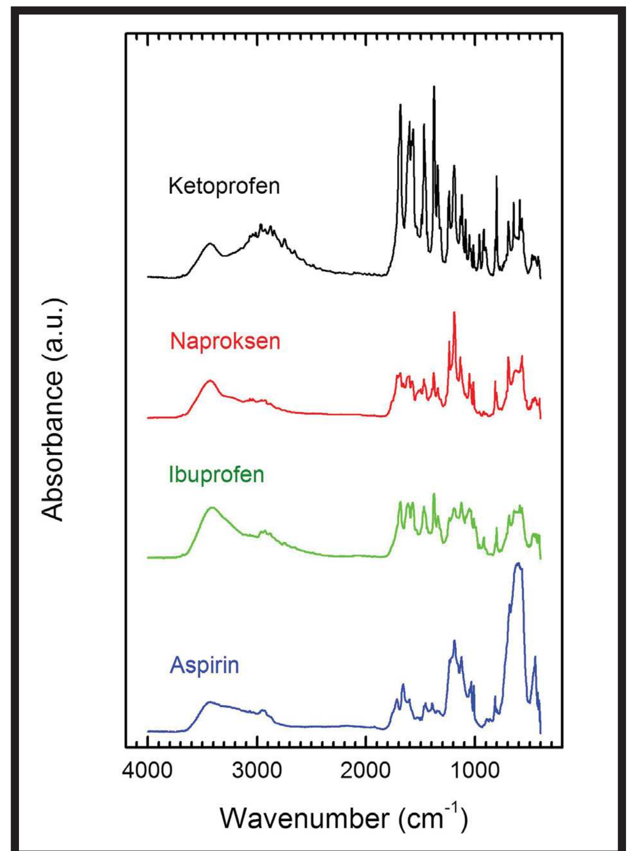

FIG. 6. FT-IR spectra of NDP with attached drugs (R-ketoprofen, R-naproksen, R-ibuprofen, R-aspirin) by amide bond with β-alanine connector- NHCO-β-Ala–NHCO-R connector.

The results of in vitro examinations with mouse fibroblasts L929 (ATCC CCL-1) show the low cytotoxicity of both method of chemical modifications (TABLE 2). The comparison between the chemical modification of nanodiamond particles by anti-inflammatory drugs without and with β-alanine linker shows slight differences of cytotoxicity although the more biocompatible seems to be modification without β-alanine. Surface modification of nanodiamond particles by ibuprofen without a connector exhibits the lowest cytotoxicity to examined cell line, and aspirin exhibits the lowest cytotoxicity (the absence of necrotic cells) for nanodiamond modified with β-alanine (FIG. 7).

FIGs 8 and 9 show the microscopic images of cells in a direct contact cytotoxicity test. The presence of living cells in contact with the chemically modified nanodiamonds indicates green fluorescence. The obtained results show very low and not statistically significant cytotoxicity of detonation nanodiamond particles, chemically modified by non-steroidal anti-inflammatory drugs in cultures of mouse fibroblasts (FIGs 8 and 9).

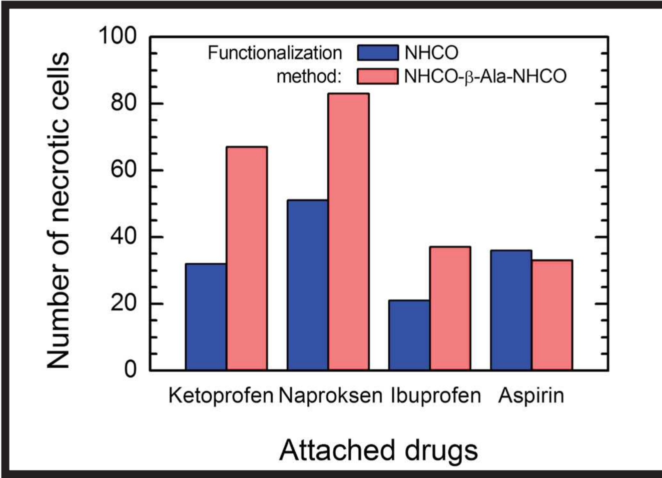

FIG. 7. Number of necrotic cells in the tested NDP samples with attached drugs (ketoprofen, naproksen, ibuprofen, aspirin) after 24 h direct cytotoxicity test for two different methods of their functionalization: "NHCO" and "NHCO-β-Ala-NHCO".

FIG. 8. Microscopic image of cells in direct contact cytotoxicity test of the following materials: A - NDP-NHCO--ketoprofen, B - NDP-NHCO-naproxen, C - NDP-NHCO-ibuprofen, D - NDP-NHCO-aspirin. Living cells positive to the FDA - green fluorescence, PI positive necrotic cells - red fluorescence, FDA + PI channel.

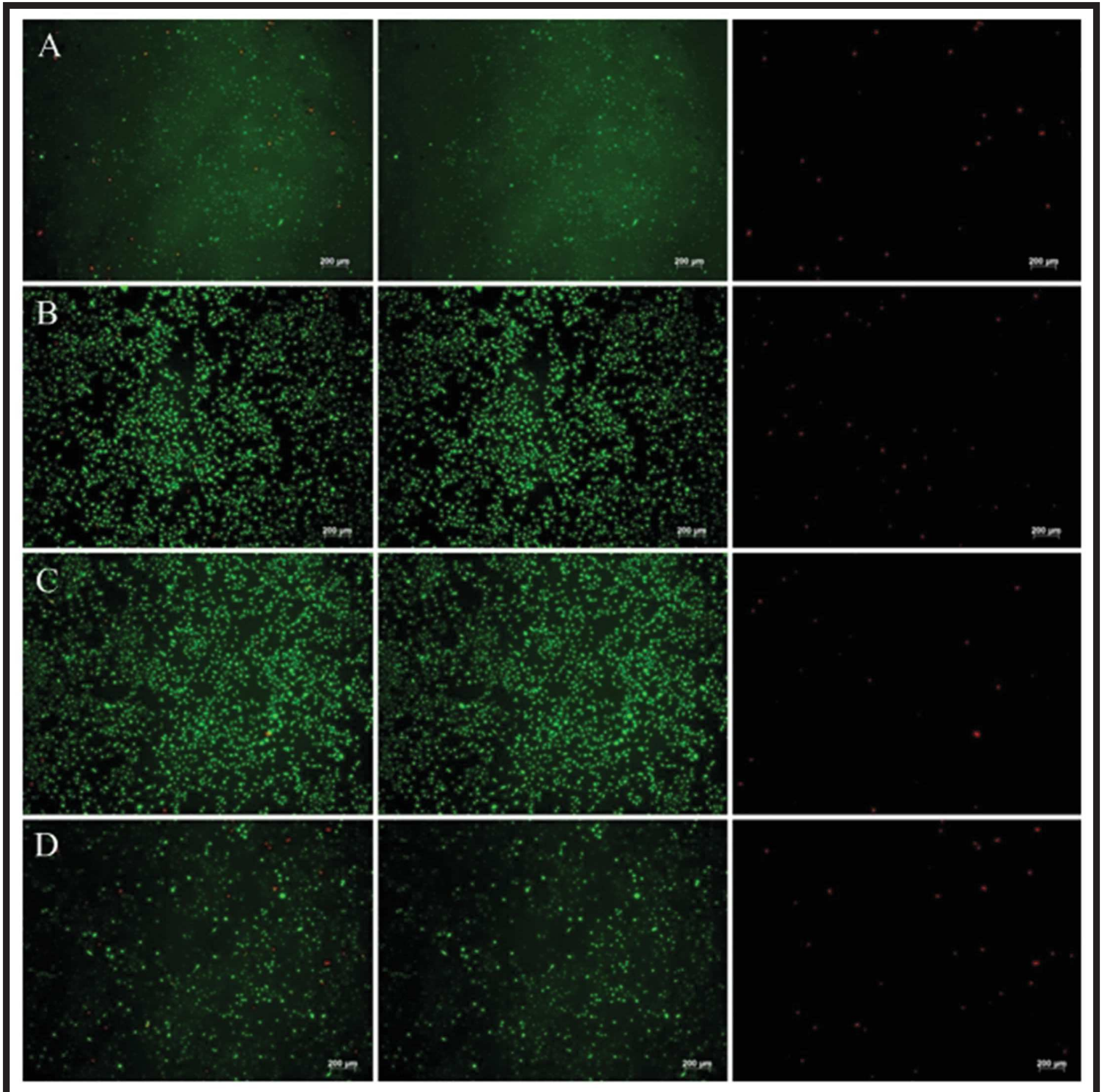

FIG. 9. Microscopic image of cells in direct contact cytotoxicity test of the following materials: A - NDP-NHCO-β-Ala-NHCO-ketoprofen, B - NDP-NHCO-β-Ala-NHCO-naproksen, C - NDP-NHCO-β-Ala-NHCO-ibuprofen, D - NDP-NHCO-β-Ala-NHCO-aspirin. Living cells positive to the FDA - green fluorescence, PI positive necrotic cells - red fluorescence, FDA + PI channel.

The number of patients with cardiovascular diseases who, due to the exhaustion of the conventional treatment opportunities, need to have the heart bioprosthesis applied constantly increases. The increasing expectations are also associated with the introduction into the clinical practice the treatment that uses the stem cells in which, apart from the proper selection of the type of the implanted cells it is very important to optimize methods for the transfer of cells into the affected area. Both, in the case of heart bioprostheses design and scaffolds for cell transfer, except for the appropriate design solutions, it is essential to use the advanced innovative biomaterials. Such materials ought to be biocompatible and should allow for proper cultivation of cells.

It is recommended that the cells after the cultivation process are characterized by high viability, the preservation of normal morphology, good growth, adhesion and migration deep into the scaffold. In the case of stem cells, it is also essential to stimulate the differentiation of the cells. Because of the increasing disparity between the number of donors and recipients, seeking for optimal materials, due to the prolonged contact with the blood, somatic and stem cells, seems to be crucial. Such materials may be based on natural biological human or zoonotic tissues; however, synthetic materials may be used as well. Both in the case of biological and synthetic materials, the normal cell interaction with the substrate is determined by the surface modification of materials.

Great hopes, due to the biological characteristics, are placed in nanodiamonds. They may stabilize, in the mechanical way, the applied surface. They may also stimulate cellular processes, particularly in the case of conjugation with the biologically active agents. Therefore, it is vital to carry out cytotoxicity test of such materials. This allows for the pre-selection of the particles for surface modification purpose. In order to do so, tests that use the model cells – fibroblasts are frequently applied. In these studies it is shown that fibroblasts in contact with the tested biomaterial particles retain their high cell viability. They also reveal proper adhesion to the substrate. This may indicate a possibility of nanodiamonds employment in applications related to the tissue engineering. The present results are very promising, however, in the future the scope of research needs to be broaden and, above all, there is a need for a strong focus on the influence of diamond powders or substrates, modified by diamond powders, on mesenchymal cells. Such studies will certainly allow for coming closer to the research model for in vivo applications in models of large animals and, in the future, in clinical studies.

The nanodiamond powders produced by the detonation method with a grain size of 2-5 nm display a high biological activity at the molecular level affecting the expression of genes responsible for inhibition of oxidative stress and carcinogenic processes [29-31]. The diamond phase content, the grain size and the method of obtaining carbon powders, including nanodiamonds, significantly affect their biological activity [31]. The functionalization of nanopowder surface increases the possibility of the controlled activity of the nanodiamond surface, e.g.: towards reducing the inflammatory reaction and antibacterial activity, and to create a biosensor that is sensitive to the presence of pathogenic bacteria in the presence of the nanodiamond [6].

The functionalization of nanoparticles is aiming to change and/or improve their biological properties. From the perspective of obtaining the modified nanoparticles, the approach based on the physical deposition of the modifying compound on a solid nanocarrier is easier. However, from the point of view of using the modified nanoparticles in *in vitro* tests such approach carries the risk of too rapid release of the physically related compounds. The sustainability improvement can be achieved through the use of covalent bonding methods between nanoparticles and the compounds of interest.

In this approach, also, it is possible to modulate the stability of the conjugates by selecting the nature of covalent bonds between the nanoparticle and the ligand. The amide bond, used in the research, formed between H_2N-NDP and the carboxyl groups of non-steroidal anti-inflammatory drugs (aspirin, ibuprofen, ketoprofen and naproxen) should ensure the relative stability and thus, the resistance to proteolytic enzymes.

The key factor assuring success in the acylation of amine groups on the surface of the nanoparticle by acid derivative (**4a-d** respectively) is the selection of an efficient condensing agent, allowing for formation of the amide bond in the amphiphilic environment. The amphiphilicity of the environment is the result of the polar character of the amine groups on the surface of nanopowders and hydrophobic nature of the NDP. In the study, as a condensing reagent, was used a quaternary salt of N-Triazinylammonium DMT / NMM / Tos^- **5**, which is an efficient condensing reagent in the synthesis of both polar and hydrophobic objects. The structure of the triazine esters **6a-d**, which are appropriate acylating reagents, was confirmed on the basis on the IR testing, where was observed a characteristic band in the range of 1750 to 1780 cm^{-1}. Another very important factor, regarding the modification of solid carriers, is the efficiency of the removal of reaction by-products, thereby eliminating the deposition of deposits in the functionalized material. The use of a triazine condensing reagents provides this aspect, since the 2-hydroxy-4,6-dimethoxy-1,3,5-triazine is easily removed by polar solvent or water extraction. The structure of final derivatives of non-steroidal anti-inflammatory drugs attached to the NDP surface was confirmed by the FT-IR analysis.

During the study we obtained NDP modified by non-steroidal anti-inflammatory drug (NSAID) in which an additional link between the NDP surface and the acid residue was inserted. As the connector the rest of β-alanine was used. The selection of the amino acid was not random. At first, we expected that the nature of the amino acid linker will improve the biocompatibility of conjugates, on the other hand, the introduction of β-amino acid residues provides resistance to proteolytic enzymes. The synthesis of conjugates **9a-d** included additionally the stage of incorporation of FMOC of the blocked amino acid and its deprotection by piperidine. The final step was the acylation of the amino group with a triazine esters **6a-d**.

Conclusions

The results of FT-IR spectroscopy proved nanodiamond's surface modification by ketoprofen, naproxen, ibuprofen and aspirin.

Mouse fibroblasts in contact with the tested detonation nanodiamond particles retain their high cell viability.

Chemically modified detonation nanodiamond particles by non-steroidal anti-inflammatory drugs reveal the low cytotoxicity towards mouse fibroblasts.

The chemical modification of detonation nanodiamond particles gives the possibility to control the surface reactivity of nanodiamonds.

Currently, it is possible to obtain the chemically functionalized nanodiamond particles containing functional groups as the linkers for bioactive molecules.

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References

[1] Y. Xing, L. Dai: Nanodiamond in nanomedicine. Nanomedicine 4 (2009) 207-218.
[2] V.V. Danilenko: On the history of the discovery of nanodiamond synthesis. Phys. Solid. State. 46 (2004) 595-599.
[3] V.Yu. Dolmatov: Modern industrial technology of reception detonation nanodiamonds and the basic areas of their use. Nanotechnics 1 (2008) 56-78.
[4] A. Krüger: The structure and reactivity of nanoscale diamond. J. Mater. Chem. 18 (2008) 1485-1792.
[5] O.A. Shenderova, G. McGuire, Nanocrystalline Diamond, in: Y. Gogotsi, (Eds.), Nanomaterials handbook. CRC Press, Taylor and Francis Group, Boca Raton, 2006, 203-237.
[6] K. Mitura, I. Gisterek, P. Ceynowa, A. Wachowicz, G. Pich, R. Woś, K. Adach: Badania mikrobiologiczne bioaktywnych proszków nanodiamentowych modyfikowanych chemicznie, in: L. Leniowska, Z. Nawrat, (Eds.), Postępy inżynierii biomedycznej, Uniwersytet Rzeszowski, Rzeszów, 2013, 157-167.
[7] K. Adach: Chemical modification of diamond nanoparticles produced by detonation method. PhD Thesis, Technical University of Łódź, 2013.
[8] P. Ceynowa, K. Mitura, W. Zinka, S. Mitura: System modyfikacji nanoproszków diamentowych (DPP) w rotacyjnej komorze reaktora plazmo-chemicznego (MW PACVD). Elektronika 10 (2014) 47-50.
[9] Ah-Y. Jee, M. Lee: Surface functionalization and physicochemical characterization of diamond nanoparticles. Curr. Appl. Phys. 9 (2009) 144-147.
[10] I. Larionova, V. Kuznetsov, A. Frolov, O. Shenderova, S. Moseenkov, I. Mazov: Properties of individual fractions of detonation nanodiamond. Diam. Relat. Mater. 15 (2006) 1804-1808.
[11] O.A. Shenderova, D.M. Gruen: Ultrananocrystalline Diamond: Synthesis, Properties, and Applications. ed. Wiliam Andrew Publishing Norwich, New York, 2006.
[12] O.A. Shenderova, S.A. Ciftan Hens: Detonation Nanodiamond Particles Processing, Modification and Bioapplications, in: D. Ho (Eds.), NANODIAMONDS. Application in Biology and Nanoscale Medicine, Springer, New York, 2010.
[13] A.P. Puzyr, A.V. Baron, K.V. Purtov, E.V. Bortnikov, N.N. Skobelev, O.A. Mogilnaya, V.S. Bondar: Nanodiamonds with novel properties: A biological study, Diam. Relat. Mater. 16 (2007) 2124-2128.
[14] V.N. Mochalin, O. Shenderova, D. Ho, Y. Gogotsi: The properties and applications of nanodiamonds. Nat. Nanotechnol. 7 (2012) 11-23.
[15] M.V. Baidakova, Y.A. Kukushkina, A.A. Sitnikova, M.A. Yagovkina, D.A. Kirilenko, V.V Sokolov, M.S. Shestakov, Ya.A. Vul, B. Zousman, O. Levinson: Structure of nanodiamonds prepared by laser synthesis. Phys. Solid. State. 55 (2013) 1747-1753.
[16] E.V. Basiuk, A. Santamaria-Bonfil, V. Meza-Laguna, T.Y. Gromovoy, E. Alvares-Zauco, E.F. Contreras-Torres, J. Rizo, G. Zavala, V.A. Basiuk: Solvent-free covalent functionalization of nanodiamond with amines. Appl. Surf. Sci. 275 (2013) 324-334.
[17 P. Wilczek, A. Lesiak, A. Niemiec-Cyganek, B. Kubin, R. Slomski, J. Nozynski, G. Wilczek, A. Mzyk, M. Gramatyka: Biomechanical properties of hybrid heart valve prosthesis utilizing the pigs that do not express the galactose-α-1,3-galactose (α-gal) antigen derived tissue and tissue engineering technique. J. Mater. Sci-Mater.M. 26 (2014) 5329.
[18] P. Wilczek , Z. Malota A. Baranska, A. Niemiec-Cyganek, B. Kubin R. Slomski, J. Nozynski G. Wilczek, A. Mzyk, M. Gramatyka, J. Opiela: Age-related changes in biomechanical properties of transgenic porcine pulmonary and aortic conduits, Biomedical Materials. Biomed. Mater. 9 (2014) 055006.
[19] P. Wilczek, R. Major, L. Lipińska, J. Lackner, A. Mzyk: Thrombogenicity and biocompatibility studies of reduced graphene oxide modified acellular pulmonary valve tissue. Mat. Sci. Eng. C-Mater. 53 (2015) 310-321.
[20] J.-I. Chao, E. Perevedentseva, P. Chung, K.-K. Liu, Ch.-Y. Cheng, Ch.-Ch. Chang, Ch.-L. Cheng: Nanometer-Sized Diamond Particle as a Probe for Biolabeling. Biophys. J. 93 (2007) 2199-2208.
[21] T.-J Wu, Y.-K. Tzeng, W.-W. Chang, Ch.-A. Cheng, Y. Kuo, Ch.-H. Chien, H.-Ch. Chang, J. Yu: Tracking the engraftment and regenerative capabilities of transplanted lung stem cells using fluorescent nanodiamonds. Nat. Nanotechnol. 8 (2013) 682-689.
[22] T. Burleson, N. Yusuf, A. Stanishevsky: Surface modification of nanodiamonds for biomedical application and analysis by infrared spectroscopy, JAMME. 37 (2009) 258-263.
[23] R.J. Edgington, A. Thalhammer, J.O. Welch, A. Bongrain, P. Bergonzo, E. Scorsone, R.B. Jackman, R. Schoepfer: Patterned neuronal networks using nanodiamonds and the effect of varying nanodiamond properties on neuronal adhesion and outgrowth. J. Neura. Eng. 10 (2013) 056022.
[24] E. Marianne, J. Mertens, J. Frese, D. A. Bölükbas, L. Hrdlicka, S. Golombek, S. Koch, P. Mela, S. Jockenhövel, F. Kiessling, T. Lammers, FMN-Coated Fluorescent USPIO for Cell Labeling and Non-Invasive MR Imaging in Tissue Engineering. Theranostics. 4 (2014) 1-10.
[25] R. Martin, P. Heydron, M. Alvaro, H. Garcia: General Strategy for High–Density Covalent Functionalization of Diamond Nanoparticles Using Fenton Chemistry, Chem. Mater. 21 (2009) 4505-4514.
[26] M. Satyanarayana, F. Vitali, J.R. Frost, R. Fasan: Diverse organo-peptide macrocycles via a fast and catalyst-free oxime/intein-mediated dual ligation, Chem. Commun. 48 (2012) 1461-1463.
[27] B. Kolesinska, K.K. Rozniakowski, J. Fraczyk, I. Relich, A.M. Papini, Z. Kamiński: The Effect of Counterion and Tertiary Amine on the Efficiency of N-Triazinylammonium Sulfonates in Solution and Solid-Phase Peptide Synthesis J. Eur. J. Org. Chem., doi.org/10.1002/ejoc.201402862, 2 (2015) 401-408.
[28] K.G. Jastrzabek, R. Subiros-Funosas, F. Albericio, B. Kolesinska, Z. Kaminski: 4-(4,6-Di[2,2,2-trifluoroethoxy]-1,3,5-triazin-2-yl)-4--methylomorpholinium Tetrafluoroborate. Triazine-Based Coupling Reagents Designed for Coupling Sterically Hindered Substrates. J. Org. Chem. 76 (2011) 4506-4513.
[29] K. Bakowicz-Mitura, G. Bartosz, S. Mitura: Influence of diamond powder particles on human gene expression. Surface and Coatings Technology 201 (2007) 6131-6135.
[30] T. Niemiec, M. Szmidt, E. Sawosz, M. Grodzik, K. Mitura: The effect of diamonds nanoparticles on redox and immune parameters in rats. Journal of Nanoscience and Nanotechnology 11 (2011) 1-6.
[31] M. Czerniak-Reczulska, P. Niedzielski, A. Balcerczyk, G. Bartosz, A. Karowicz-Bilinska, K. Mitura: Biological properties of different type carbon particles in vitro study on primary culture of endothelial cells. Journal of Nanoscience and Nanotechnology 10 (2010) 1065.