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USE OF NANODIAMONDS IN BIOMEDICINE

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The aim of this work is to summarize the literature data concerning the strategy of creating of methods of efficient nanotherapy and targeted drug delivery. It was shown that the developed methods of post-refining, control surface cleanliness and creating different types of hydrophilic surface of nanodiamonds provide ample opportunities for high-quality surface functionalization of organic compounds.

Modern direction of nanomedicine is creation of complex biocompatible nanomaterials with antitumor activity that promote the targeted drug transport in the place of localization of pathological process. To provide targeted drug delivery C_{60}-fullerens, nanoporous silica, carbon nanotubes and nanodiamonds are widely used. The combination of nanodiamonds with other nanoparticles and their association with drugs can be considered as a promising strategy to overcome tumor resistance to the drug action. It can be suggested that nanodiamonds coated with polyethylene glycol is the way to create the stable and selective carriers of new types. They have to be biocompatible, stable, possess higher dispersion in biological media and avoid recognition by the immune system. Such composites effectively enter the cell cytoplasm via clathrin –mediated endocytosis. Hybrid nanocarriers will become the basis for the targeted and controlled release of high concentrations of antineoplastic chemoterapeutic agents within the cytosol of the cancer cells with minimal non-specific binding and toxicity towards normal cells.

Key words: nanodiamonds, hybrid nanocarriers, biomedicine.

The quick development of nanotechnology was related with synthesis and usage of wide range of nanomaterials. Among them there are main crystalline allotropic forms of carbon such as fullerenes, nanotubes, graphenes, carbin, nanodiamonds (ND), they represent the important source for biomedical applications [1–7].

Over the last decades, carbon nanomaterials (CNMs) have attracted a great deal of attention due to their unique structure and remarkable electronic and mechanical properties. These materials have shown promising applications in electronics, energy conversion and storage, and biomedicine etc. [8, 9]. Fullerenes and carbon nanotubes are used for the development and synthesis of new nanostructural materials with corresponding biological properties [4–6, 9–13]. Creation of the materials possessing high biocompatibility, low toxicity and high specificity accumulates the achievements of nanobiotechnology and uses knowledge of Chemistry, Physics, Biology, Medicine and materials. The practical tasks are aimed at study of functional properties of nanomaterials and the development of new methods. Many problems of the modern nanomedicine have been studied by Ukrainian scientists [3–6, 12–14].

Some synthetic and natural nanoparticle carriers were developed to create fluorescent images of biological objects and to provide drug transport [12, 15]. These complexes consist of polymer carbon nanotubes [12], peptide conjugates, liposomes, micelles, dendrimers, polyelectrolyte, gold nanoparticles, magnetic nanoparticles, semiconductor quantum dots etc. [15].

Recently, the progress was achieved to use CNMs in biotechnology. For example, it concerns the solubility and biocompatibility of carbon nanotubes. For this purpose carbon nanotubes with different biologically active compounds were modified. Prototypes of biosensors were projected and developed [16]. In case of CNM the fullerenes and carbon nanotubes were used [9, 17–30]. Nanotubes, fullerenes and graphene have rather essential toxicity to cancer cells [31], that was the reason of their using as antitumor drugs [3, 5, 11–13, 22, 24, 27]. However, their implement is limited to technological peculiarities, i.e., it is necessary to perform additional treatment

of the material and to use special methods for it. The estimation of biocompatibility needs complicated methods to check toxicity of the material and to detect the further complications [7, 9]. However, the area of using of different nanoparticles has constantly been increased.

Among CNM the special attention has to be paid to ND (solid particles or films), which become new material in nanomedicine. They have high affinity towards biomolecules and possess the lack of toxicity, chemical inertness, high reliability and possibility of different surface functionalization [7, 15]. As compared with other carbon nanomaterials, such as carbon nanotubes, which toxicity was shown in some investigations, ND may be considered as a material for biomedical using in physiological systems [9]. As it was shown by biocompatibility investigations ND are not toxic for different types of cells [7, 9, 32].

Using ND in biomedicine

Nanodiamonds belong to an important class of materials which possess unique structural characteristics and unusual mechanical, electrical, thermal, optical and biological properties [33]. The systems, which are created on the base of ND structure, can be used in biomedicine as luminescent and stable markers due to their physical properties and biocompatibility [9]. Besides, they may be used to keep track of malignant cells by fluorescence without photobleaching [33]. Fluorescent nanodiamonds (FND) are used to track one particle in the cell even in small organisms such as *Caenorhabditis elegans* [8, 34]. Recently ND have been widely investigated in connection with their using in biology and medicine taking into consideration their physical and chemical properties such as an extremely large surface, biocompatibility and a small size (2–10 nm) of their ultrafine diamonds (UD) [8, 34].

Carbon atoms, which are present on the surface of ND, unlike the atoms which are within the scope, contain free valence electrons and therefore are able to join atoms of other elements. The presence of active groups of atoms facilitates the chemical modification of the surface and gives the possibility to adjust the properties of ND [32].

Atoms of ND surface can be oxidized, reduced or functionalized by different agents to provide the surface with hydrophilic or hydrophobic properties [35]. All above mentioned make possible the reliable functionalization of the surface by organic compounds including biomolecule immobilization — peptides or DNA [36], medicaments [7, 14], optical marks etc [32].

After cleaning the surface of UD is usually covered by hydrophilic functional groups –OH and –COOH, and the stable dispersion in water (100 μg/ml) is achieved by mild sonication for 30 minutes [37]. Some aprotic solvents, such as methylpyrrolidone and dimethylsulfoxide provide better dispersion stability of ND than water [9].

Unlike other nanomaterials, availability of UD is not a problem for large-scale using due to well-established low-cost industrial detonation synthesis [9]. That is why they are used in various branches of science, medicine and technology [7], and the interest in ND has grown steadily over the last decade [14, 32].

Nanodiamonds can be used as materials to coat surgical instruments and implants for biomedicine. Due to their hardness, chemical inertness, thermal conductivity and low cytotoxicity coverages with ND are promising for the creation of medical implants in cardiovascular surgery, as well as coverages of certain components of artificial heart valves because of their extremely high chemical inertness, smooth surface and adequate adhesion of the coating to the substrate [7].

ND particles have a large surface area and high adsorption capacity [7] in particular, they can absorb water up to four times its own mass, which allows to place on their surface various pharmaceutical preparations.

The specific surface of the ND is about 300 m^2/g, so they are excellent sorbent materials like activated carbon [35]. With their high adsorption, ND may be considered as potential drugs for the normalization of the gastrointestinal tract, excretion of adverse and toxic compounds (metabolic products, heavy metals, radionuclides). They can also be used for treatment of vascular diseases and as effective remedies to overcome the effects of skin diseases and burns [35].

Magnetic resonance imaging (MRI) is a noninvasive medical imaging technique with the introduction of contrast agents into circulation for detailed images of internal structures in the body. Gadolinium (Gd^{3+}) is often used for contrast in MRI, but its effectiveness can be improved by a combination with ND.The complex Gd^{3+}–ND as a contrast agent for MRI [38] has a better contrast with the image resolution of a 10-fold increase compared to the image obtained with "pure" gadolinium.

ND were used as fluorescent biochip prototypes, because they preserved catalytic

activity of enzymes in the systems where the main element was made up of particles of detonation ND and bacterial obelin and luciferase [39]. UD were also applied for the immobilization of antigens due to their large surface area and lack of toxicity. It was shown that immobilization of ND helps to support peptide conformation, facilitates antibody activity and enhances an immune response. Besides, preparations of ND-complexes have no influence on the development of cancer [40]. The possibility of visualization of objects with UD is the result of the latest achievements in the field of biomedical using of ND [7, 37, 41–49].

FND possess the negatively charged nitrogen-vacancy centers, (N-V) which provide the fluorescence abilities of these compounds [14, 32, 50]. Intensive fluorescence may be obtained by surface modification of ND [51, 52]. So, hybrid structures can be created on the base of FND with fluorescent compounds. Hydrophobic blue fluorescent ND were synthesized by covalent binding of octadecylamine on their surface. The obtained preparation was easily dispersed into hydrophobic solvents forming a transparent colloidal solution. It can be used when the stable dispersion of ND should be made [52; Fig. 1].

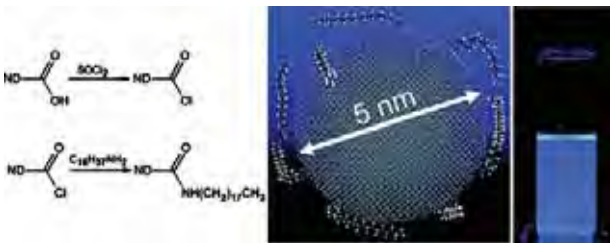

Fig. 1. **Schematic of synthesis and fluorescence of hydrophobic ultrafine diamonds when their surface was modified with covalently conjugated octadecylamine [52]**

Fluorescence of UD surface modified with octadecylamine opens the possibility to use the composites (UD-octadecylamine) as non-toxic fluorescent nanoparticles to create biomedical images of cellular membranes and other hydrophobic components of biological systems. Similar modifications of the surface can be applied for other carbon nanoparticles [52].

Modification of the chemical surface and incorporation into the polymer molecule is used in many ND technologies. Such composites can be utilized as platforms for biological and medical investigations to transfer peptides and genes and as the carriers for chromatography. Ethylenediamine, which is covalently conjugated with carboxylated surface of ND, creates a composite with epoxy, where UD particles (5 nm) are covalently incorporated into polymer matrix (Fig. 2). The obtained composite (UD-epoxy) had a 3-fold greater hardness compared to other composites where ND were not covalently conjugated with matrix [53].

Biotinylation is also simple, widespread and efficient method for covalent functionalization of UD [54; Fig. 3].

New material opens the way to covalently bonded diamond bioconjugates for labeling, drug delivery, and other applications. The images of silanized and biotinylated samples obtained by high-resolution electron microscopy (Fig. 4) showed that the material consists of small particles of ND of uniform size where there is practically no graphite coating. UD present in the form of small agglomerates [54].

Recently, NDs are applied as the base of therapy for mediated delivery of water-insoluble pharmaceuticals [7].

Nanodiamonds have been investigated extensively for drug delivery and sustained release of anticancer preparations [37], nucleic acids [55] and insulin [46]; they may be applied in tissue engineering, other systems of of drug delivery [37, 41–49] and as biomarkers [34].

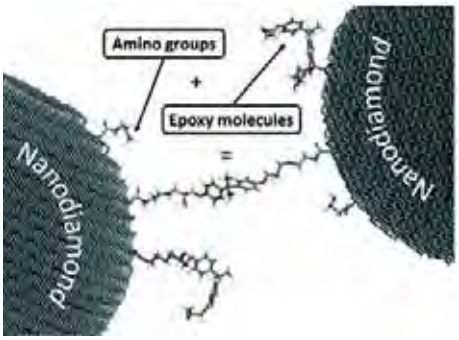

Fig. 2. **Schematic of creation of the composite where ND paricles (5nm) are covalently incorporated into polymer matrix:**
Carboxylated surface of ND modified by amino groups is conjugated with ethylenediamine and creates a composite with epoxy [53]

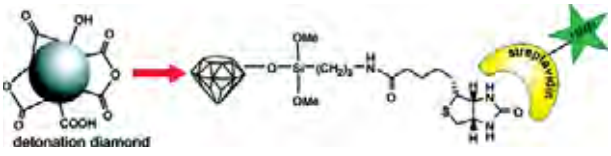

Fig. 3. **Surface of ND modified with (3-aminopropyl) trimethoxysilane followed by covalent attachment of biotin:**
the activity of the complex was tested with horseradish peroxidase-labeled streptavidin [54]

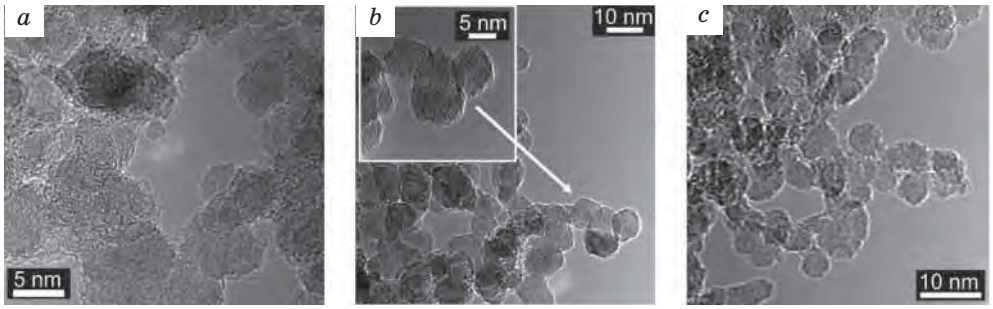

Fig. 4. **Image of detonation nanodiamonds using high-resolution electron microscopy:**
a — lattices of diamond are clearly visible, and primary nanoparticles have a fairly uniform sizes and form of distribution;
b — aminopropyl-silanized nanodiamonds (insert shows an increase of some particles);
c — biotinilated nanodiamonds.
Bright lines that surround the particles are the result of defocusing that was used for the better contrast. The size of lines is 5 and 10 nm [54]

Gene therapy is prospective for treatment of many diseases including cancer, cardiovascular diseases, diabetes and many others. ND are promising carriers for a new generation of therapy with proven potential. It was created the particles of ND with modified surface which can transfer DNA to mammalian cells [56].

NDs with the low molecular immobilized polyetilenimine (PEI 800), which was covalently connected by amino groups to the surface of the diamond, were 70 times more efficient in transferring DNA than conventional standard techniques transporting genes [55; Fig. 5].

The new method can affect the development of many aspects of nanomedicine. However, the creation of an effective system for transporting genes into cells that would be effective and safe is a challenge. The importance of ND (considered as the object of new technology for gene transferring) combines the efficiency of transportation together with significant biocompatibility [55].

The stability of the structure and the ability to provide the surface modification with various chemical groups makes ND a notable material for carrying biologically active drugs and markers into the cells. Adsorption is the main mechanism, most commonly used for drug download on UD. It was demonstrated the importance of ND for transportation and sustained release of anticancer drugs. Using doxorubicin (dox) and polymyxin as the examples it was shown that physical and chemical adsorption on UD depends on the chemical properties of adsorbent surface and the quality of cleaning [37].

ND modifications

Long-term investigations of ND showed the significant difference of the surface properties of UD concerning the micropowders of static synthesis. These properties depend on the nature and the amount of adsorbed functional groups. There is significant amount

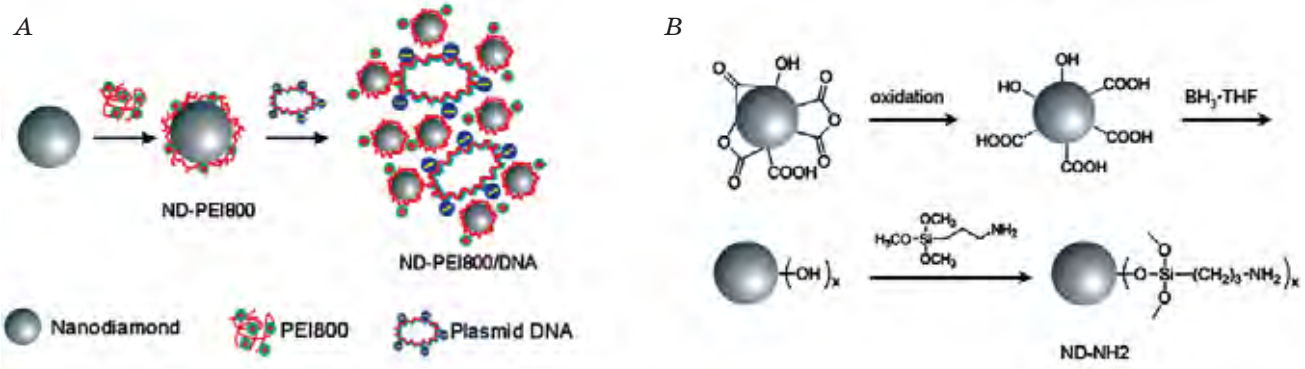

Fig. 5. **Schematic of the process of creation of hybrid material of ND — PEI 800-DNA (*A*), which transfers DNA into the cells and modification of nanodiamond surface (*B*):**
polyethyleneimine (PEI 800), nanodiamond modified with NH_2 (ND-NH_2) [55]

of water and OH-groups on the surface of UD, there are also carbonyl and carboxyl groups. Hydrophilicity of NDs is rather significant; it is 2 times higher according to the absolute value compared to this one in case of diamonds of static synthesis [56]. NDs created at the different firms may possess diffrerent properties, because using of thermal or physical and chemical treatment affects the composition of their surface. So, thermal treatment of powder samples in argon leads to significant loss of OH-groups [57]. The surface charge of the particles (ξ-potential) of detonation diamonds is negative and it is ten times higher than that of powders of static synthesis [56]. Using the methods for additional purification of NDs it is possible to obtain the powders which surface activity is different from that one of untreated surface in 1.5 times. Thermal treatment of the powders increases their active surface in 4.5 times [57].

Diamonds can be modified by the introduction of some elements in their composition.There are boron- or nitrogen-doped diamonds. A thin layer of polyaniline/poly (acrylic acid) composite polymer film could be electropolymerized onto the diamond surface. The diamond is boron-doped. The carboxylic acid residues in the polymer film act as binding sites for DNA attachment [58]. Nitrogen is always presents in nucleus of ND in sp^3-hybridization. The main reason of nitrogen-doped UD is obtaining of stable photoluminescent properties. The content of nitrogen depends on precursors, which are used as a part of the explosive compounds: inclusion of melamine leads to a 2–3 fold increase in the content of nitrogen of UD [59].

Modern methods of ND purification

The knowledge of the detailed structure of UD particles is the necessary condition to prepare them for chemical functionalization. Fourier transform infrared spectroscopy, nuclear magnetic resonance and X-ray photoelectron spectroscopy are the main methods to study these particles [60]. The reduction reaction helps to create chemically functionalized UD enriched hydroxyl and hydroxymethyl functional groups. As it was shown by infrared spectra analysis and quantum chemical modeling the vacuum cleaning of the sample (to remove water and volatile contaminants) is a necessary step to reach full cleaning of the material and to carry out the precise definition of the surface structure [60]. The presence of a great amount of graphite in UD essentially limits the application of such nanomaterials. The main demands for cleaning of ND surface are low content of sp^2-carbon and maintaining of the small size of the agglomerates. Many methods were developed for ND cleaning. Thus, water-soluble NDs were obtained. For this purpose the graphite surface was reduced using sodium in liquid ammonia and functionalization of the surface was carried out with the carbon acid [61]. The main task is the development of cheap, ecologically friendly technologies [61]. For example, the simple and ecologically friendly technology with using of oxidation of UD by air leads to the selective removing of sp^2-carbon, which is bound with their surface [62]. The modern methods of UD cleaning use oxidation by oxygen or ozone that makes possible to obtain UD with the same characteristics from different producers. The purification of detonation soot using ozone treatment is one of the most efficient methods to obtain UD. NDs cleaned by ozone have a number of features such as high density of oxygen-containing groups, high acidity, high oxygen content, low content of sp^2-carbon and significant content of small particles [63]. These NDs possess high stability of colloidal system in a wide range of both — pH (2–12) and temperature, particularly from the boiling point to the freezing point. After freezing and thawing organosol retains colloidal stability [63]. On the base of modified NDs the hydrosol was developed. It has a high stability of colloidal particles, and dry powder can be converted into hydrosol by the simple adding of water; sonication is not needed [64].

To understand these peculiarities of UD oxidation by ozone, some researchers studied the nature of surface functional groups formed during oxidation. It was shown that carbon anhydrite groups dominate on the surface of UD oxidized by ozone. According to the results of electron microscopy, ultrafine diamond treated by ozone demonstrate small amount of amorphous carbon on the surface of the diamond crystal (Fig. 6).

The temperature profile of desorption of volatile products, their mass balance and other studies showed that the decomposition of carboxylic anhydride groups on the surface of UD during the heating occur in two different mechanisms that can be controlled [63]. Their existence is probably connected with different binding energy of these groups on the crystallographic faces of ND particles [63].

It is shown that sp^3/sp^2-hybridized carbon is oxidized at different rates in the temperature range 375–450 °C. Non- diamond carbon can be

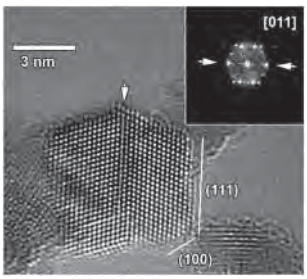

Fig. 6. **Electron microscopy image of ozone-purified ultrafine diamonds with minimal presence of sp^2-carbon [63]**

removed selectively, oxidation of sp^2-hybridized carbon occurs in a narrow temperature range 400–430 °C with no or minimal loss of the diamond. There is an increase in the ratio of sp^3/sp^2 to two orders of magnitude after UD oxidation on the surface. Some studies indicated a high purity of 5 nm ND particles with oxygen-containing functional groups [63]. In contrast to modern methods of cleaning the oxidation process in the air does not require the use of toxic and corrosive chemicals, catalysts or inhibitors and opens opportunities for widespread use of UD [63]. Raman spectrum of oxidized UD at 400 °C shows a significant activation signal of the diamond [Fig. 7].

The ratio of intensities between the group of the diamond and the G-group of graphite in the Raman spectrum of UD reaches the maximal value within the temperature range 400–430 °C. It indicates the best conditions of cleaning, when the content of diamonds is maximal and the content of amorphous and graphite carbon is minimal.The control of the surface purity and the ability to change the size of the particles are important for any nanomaterials. Oxidation can "burn" carbon and lead to uncontrolled changes in the particle size, but the method developed in [34] makes possible to reduce successfully the size of NDs. Using two commercial powders of NDs two stages of the oxidation reaction were separated in time: oxygen chemisorption (I) and desorption of CO and/or CO_2 (II) [Fig. 8; 34].

This technique makes it possible to effectively control the process of oxidation, which is regulated by repeating steps of chemisorption / desorption, removing layer after layer of sp^3-hybridized carbon. It was shown the gradual decrease in the size of NDs. In accordance with the reduction in the size after oxidation, the surface area of nanopowders is cleared not only of adsorbed substances and graphite layer, but it is cleared with sp^3 carbon and covalently attached to it molecules that gives additional opportunities for the use of ND in nanotechnology [34].

Strategy of creation of universal nanotherapeutical materials

Passive target delivery of nanocarriers is based on the increased vascular permeability of the tumor and reduced efficiency of duct

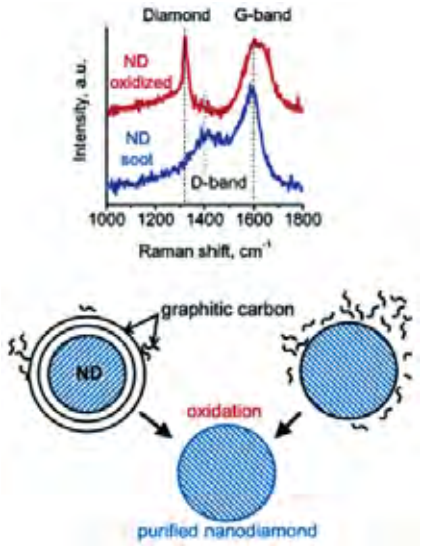

Fig. 7. **UV (325 nm)-raman spectra:** G-band of graphite (1 600 sm^{-1}), the diamond peak is at ~1 325 sm^{-1}. The spectrum of untreated raw powder with the high content of non-diamond carbon (see below). Raman spectrum obtained by UD oxidation at 400 °C for 5 hours shows a significant activation signal of the diamond phase (at the top) [63]

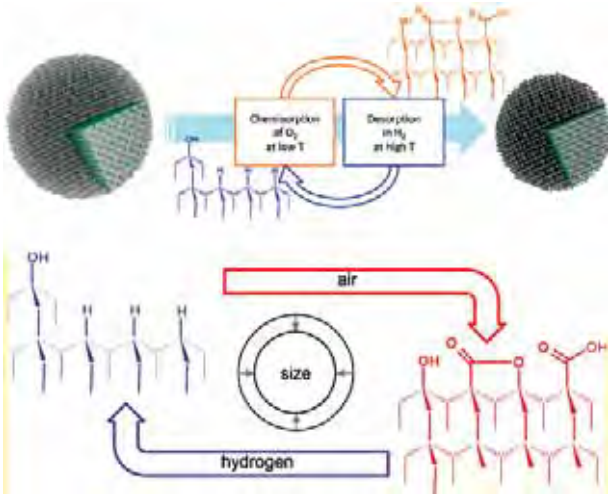

Fig. 8. **Schematic of the layer-by-layer oxidation process for controlled size reduction of NDs:** Oxygen chemisorbed on the ND surface. In a subsequent step, the chemisorbed oxygen is desorbed at elevated temperatures. Hydrogen is used to preserve sp^3-hybridization. The chemisorption and desorption steps are repeated in cycles, leading to a controlled burn off. The sectors are cut from molecular models of NDs to show their interior [34]

lymphatic tumor vessels. It leads to the direct accumulation of carriers at the place of tumor location, but because of the absence of specific interaction with tumor cells, the success is not guaranteed.

Although simple accumulation of nanocarriers with medicine provides better results than just administration of anticancer drugs in the bloodstream, the passive target transportation reduces the therapeutic efficacy of nanocomposites that results in little drug effect and induction of multiple drug resistance (MDR) [65, 66].

Today's trend of nanomedicine is to create new biocompatible complex nanomaterials with antitumor activity. These materials have to facilitate target transport of drugs in the place of localization of pathological processes and enhance antitumor action in combination with traditional chemotherapy, reducing the toxic effects in the human body [12, 65]. C_{60}-fullerenes [5, 12, 13, 19–22, 24–26], nanoporous silica [65], carbon nanotubes [4, 6] and NDs [7–9, 15, 33, 37, 42–46, 48] are promising biomedical preparations for targeted delivery of drugs.

Nanocomposite delivery in local place gives great potential for reducing of the concentration of chemotherapeutic drugs in healthy and unaffected tissue areas. This helps to reduce side effects. It is possible to create multifunctional structures on the base of nanoparticles. These structures will facilitate target drug delivery and reduce drug complications [9]. The encapsulation of drugs to nanocarriers selectively targeted malignant cells, together with transportation of different combinations of unique drugs can significantly mitigate the side effects of traditional chemotherapy [65].

Target nanocarriers must be specific, stable and have a high capacity for connection and transfer of various substances. There is an approach according to which porous nanoparticles are covered with lipid bilayer that synergistically combines the properties of liposomes and nanoporous particles. Such hybrid nanoparticles modified with targeted peptide bind to cells of human hepatocellular carcinoma and have a 10 000 times greater affinity for it than to hepatocytes, endothelial cells or immune cells [65]. In addition, hybrid nanoparticles can be loaded by combinations of therapeutic agents (drugs, small interfering RNA, toxins [65], complexes of fullerene C_{60} with cytostatics [12, 13, 19–21]) and diagnostic agents (fluorescent objects). Modification is needed to enhance chemotherapeutic action of nanoparticles. It will facilitate endosomal release and accumulation of individual substances in the cell nucleus. Significant potential of nanoporous core with a large surface in combination with efficiently stabilized lipid bilayer provides the possibility of creating of universal nanotherapeutical materials. They are easily loaded with a drug mixture, which targetly penetrates inside the cells and kills malignant cells, such as human hepatocellular carcinoma that is resistant to appropriate medications [65].

Nanocarriers have to be internalized, so for the best strategy of target drug transportation the investigators use ligands, in particular peptides [65] that specifically interact with receptors on the cell surface and facilitate the binding of nanocarriers to the membrane of target cells [67]. The multiple copies of target ligands that can conjugate with nanocarriers are used to maximize selectivity and therapeutic efficiency [65, 68]. This enhances the affinity [65, 69] and provides more efficient ways of drug transportation through receptor-mediated internalization that help bypass MDR mechanisms [65, 70]. Surface modification of nanocarriers with hydrophilic polymers (such as polyethylene glycol (PEG)) increases circulation time due to reducing the interaction with peptides of serum and slow uptake by phagocytes [65].

Hybrid nanoparticles due to the large surface area (>1000 m^2/g) and porosity of the SiO_2-cores possess higher capacity for doxorubicin (as well as for therapeutic and diagnostic agents) than similar sized liposomes and they release about 90% of encapsulated biologically active Dox directly in cells after endocytosis [65].

Fusion of liposomes with spherical large surface, nanoporous SiO_2-core [65, 71], further modification of the lipid bilayer with multiple copies of target peptides and covering the entire structure of nanocarrier by PEG significantly improves capacitive properties of carriers, their stability and selectivity. Such hybrid multicomponent nanoparticles perform aimed transportation and controlled release of high concentrations of multicomponent mixtures within the cytosol cancer cells [65].

The core of the nanoparticle is easily loaded with multicomponent chemotherapeutic mixture [65] by simple soaking of nanoporous core in a solution of corresponding compounds (eg DOX, 5-fluorouracil and cisplatin, which are particularly effective against MDR). Then, the core is coated with the bilayer lipid membrane. The target peptide SP94 is added to the membrane to provide target transportation [Fig. 9; 65].

Targeting and fusogenic peptides are chemically conjugated to phosphatidylethanolamin (DOPE or DPPE), present in lipid bilayer at 1–5 wt%, by a heterobifunctionalcrosslinker with a PEG spacer arm (n = 24). The lipid bilayer, composed of either fluid (DOPC) or non-fluid (DPPC) zwitterionic phosphatidylcholine lipids with 30 wt% cholesterol, is further modified with 5 wt% PEG-2000 PE to enhance colloidal stability and decrease nonspecific interactions.

Targering nanohybrids possess high specificity, high capacity for drug transportation and greater stability. Synergistic combination of materials and biophysical properties enables high delivery efficiency and enhanced targeting specificity with a minimal number of targeting ligands [65]. Multicomponent hybrid nanoparticles reduce the selective cytotoxicity to malignant cells, limiting undesired toxicity to intact hepatocytes. This combination causes the death of tumor cells Hep3B with saving more than 90% of viable hepatocytes [65]. Nanoporous core can be adapted for releasing of uncapsulated substance within 24 hours or even several weeks, and the lipid structures can be combined with different ligands, including peptides, antibodies or glycoproteins to enhance specific affinity for target cells [65].

Along with SiO_2 [65, 72, 73] UD and composites based on them are considered as promising and universal platform for transporting nanotherapeutical agents in oncology [7–9, 14, 33, 37, 53]. Thus, ND and UD and have been used in several medical programs and investigations (related to the interaction

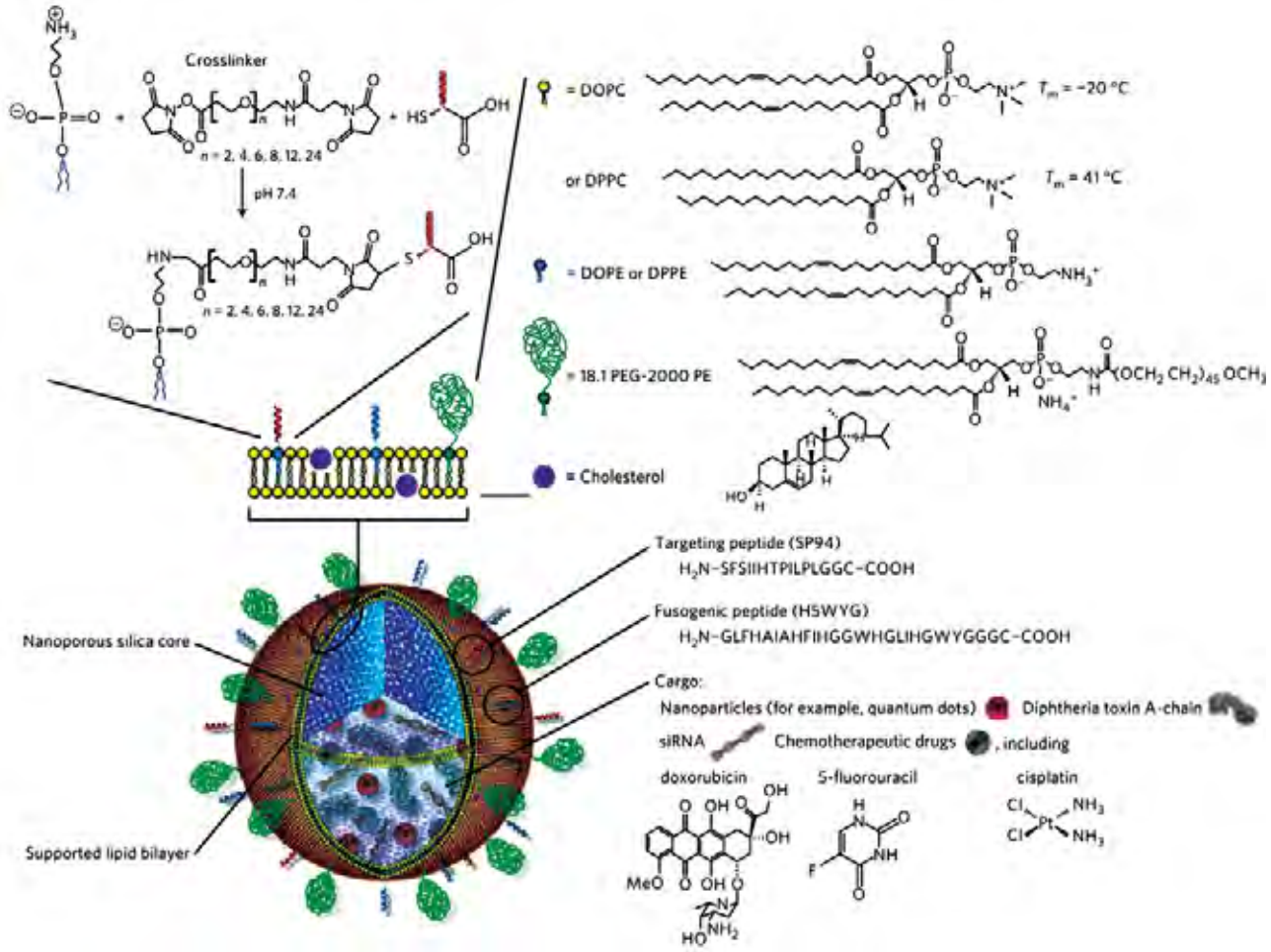

Fig. 9. **Schematic illustration of the nanoporous particle-supported lipid bilayer:**
It was shown the disparate types of therapeutic and diagnostic agents that can be loaded within the nanoporous silica core, as well as the ligands that can be displayed on the surface of the lipid bilayer [65], where
(DOPC) —1,2-dioleoyl-sn-glycero-3-phosphocholine;
(DPPC) — 1,2-dipalmitoyl-sn-glycero-3-phosphocholine;
(DOPE) — 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine;
(DPPE) — 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine;
(18:1 PEG-2000 PE) — 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]

between ND and biological objects) which were carried out to develop effective systems of drug delivery. ND can tightly bind different molecules and efficiently guide the drugs adsorbed on their surface, inside the living cells. For example, DOX was successfully applied for UD functionalization (2–8 nm) and it was introduced to mice macrophages and to human colorectal cancer cells, preserving their activity. UD were considered as non-toxic to HT-29 colon adenocarcinoma cells, although ND functionalized by DOX can cause the death of these cells [9].

For therapeutical using of NDs their potential danger to human and other biological systems should be established. Such investigations should reveal the optimal using of and prevent possible complications and adverse effects with a clear understanding of the benefits of ND using.

The previous investigations indicate excellent biocompatibility of NDs in different cell lines without significant cytotoxicity [7]. NDs easily enter and are accumulated in the cells. Carboxylated NDs are non-toxic to human lung epithelial cells and normal fibroblasts [40].

Biocompatibility was also investigated at FND (size particle was 100 nm) in kidney cell culture. The low cytotoxicity to human 293T cells was revealed. These FND were introduced into the cell by endocytosis, but their delivery to the nucleus was limited [74].

Some experiments were carried out for 96 hours on the surface modified with cations (or without modification) with macrophages, keratinocytes, cells of neuroblastoma and pheochromocytoma PC 12. It was shown the absence of toxicity and biological compatibility of NDs with these cells [2, 31]. In addition, NDs do not produce reactive oxygen species. Cells grown on the substrates, created with the addition of ND, had no existing changes compared with control [2].

DNA-damaging action of NDs was studied during their incubation with embryonic stem cells. Oxidized NDs were more toxic than those who had restored surface. So, the studied action directly depended on the specific chemical composition of the surface. DNA damages caused by the two forms of NDs were much less serious than those that were caused with multilayer carbon nanotubes [75].

It was concluded that the decisive factor ND action on cells were active functional groups on the surface of nanoparticles, but not their diamond component. It was noted the minor drug toxicity that was reduced by a corresponding change in the chemical composition of ND systems [75]. Although preliminary results are encouraging, we should continue investigations to determine the possible UD cytotoxicity, including long-term effects on cells or animals, and to prevent and eliminate the possible toxic effects created by nanocomplex [7].

NDs can be used as the platforms for future biochips and biosensors, as they have the required mechanical, thermal and chemical properties compared to other surfaces. Comparing the nanocrystalline diamond surfaces with other that are widely used for the immobilization of biological objects, including gold, silicon, glass and glassy, investigators made a conclusion that in many respects diamond is unique.

It has high stability, while the gold-thiol surface, for example, are sensitive to oxidation, which leads to the dissolution of connected layers [36].Chemically modified surface involving Si-O bonds degrades in the alkaline environment. Crystalline silicon and SiO_2 surface covered by amines are subjected to degradation [36]. Taking into consideration all above mentioned, we conclude that materials from ND can serve as platforms for controlled multi-functionalization and delivery of a number of therapeutic compounds, but for widespread using of UD we need high-quality raw material, which includes ND with different surfaces with constant characteristics.

Background for general using of ND for biotechnology

NDs with fluorinated surface, compared to oxidized one, possess better solubility in polar organic solvents such as alcohols and tetrahydrofuran, and have reduced agglomeration of particles. The developed method provides an efficient chemical modification of NDs that is the base of their using in medicine. Fluoride-ND was used as a precursor for preparing of functionalized NDs [76].

To create different hydrophilic types on UD surface and to control its purity the following method has been developed. Nanodiamond powder was designed as **ZH** (the supplier was **Z**hongchuan **H**eyuan, China). The adsorption on purified and surface modified NDs was studied. Purification was done by oxidation of NDs in air at 425–430 °C for 2 hours [37], that led to the formation mainly –COOH-groups, they were designed as ZH-COOH to emphasize their surface chemistry. Aminated NDs were produced by covalent binding ethylendiamine to –COOH; these modified NDs were designed as ZH-NH_2 [54].Those chemical modifications produced the same surface chemistry on

powders from different suppliers making them almost identical from the standpoint of adsorption. Infrared spectra of ZH, ZH-COOH and ZH-NH_2 are shown at Fig. 10 [37]. It is clear that all modifications possess their own infrared spectra.

The study of chemicotherapeutical preparations using ND is the subject of many investigations [37, 41–45, 49]. To understand the absorption of drugs used as a model DOX and polymyxin were used as a model. Adsorption of DOX was studied on ZH, ZH-COOH and ZH-NH2 [37]. In the experiments with DOX adsorption they obtained per 1 g of diamond powder on the different modified surfaces the following values: ZH — 10.49 mg/g, ZH-COOH — 87.36 mg/g, ZH-NH_2 — 288.4 mg/g [37]. For the diamonds obtained from another supplier, the polymyxin adsorption was the following (per 1 g of diamond powder): the raw — 92.3 mg (surface with significant amount of $-CH_2$ and CH_3 groups) and for covered with $-NH_2$-groups — 53.02 mg [37]. Method developing of controlled purity for various types of surfaces [37, 76] is a significant step towards the extended use of UD in nanotechnology. Creating drugs of different surface chemistry [37, 77] researchers used mechanisms of absorption and covalent binding, depending on the specific development of the system of drug delivery.

Thus, different surfaces of ND can be used for corresponding models of drug delivery. Changing the composition of the surface you can choose the necessary surface for drug adsorption [37]. The results of adsorption of NDs on the chemically different surface for DOX and polimyxyn are promising [37].

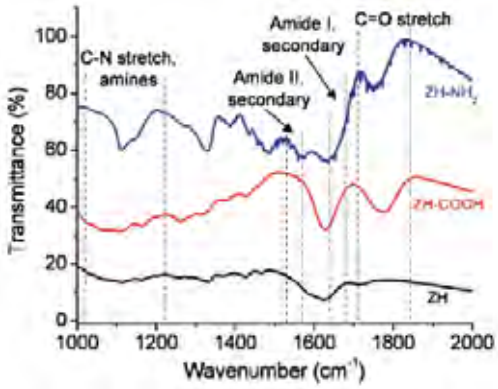

Fig. 10. **Infrared spectra of different modifications of ND surface (ZH), ZH-COOH, ZH-NH_2, (ZH = ZH-OH):**
ZH-NH_2 — diamond modified with $-NH_2$; ZH-COOH –diamond modified with–COOH-groups; ZH — primary diamond with mainly chemically conjugated OH-groups and/or adsorbed on the surface of the diamond (ZH) [37]

In order to design a specific model for drug delivery we have to study and provide the enhancement of drug adsorption on the ND surface [37]. The mechanism of adsorption / desorption is the first choice for loading drugs on the nanocarriers. This is due to its relative simplicity, versatility, minimal changes in the drug structure (without changing of the chemical structure of the drug molecule, unlike covalent binding). For example, adsorption of poorly soluble drugs on the surface of dispersed particles of biocompatible ND is an important method to overcome their poor bioavailability, which is a problem for many drugs [37]. Optimization of drug adsorption on the carriers accelerates the production of multifunctional complexes of the nanoparticles. Taking into account the dosing of many chemotherapy drugs is essential to reduce the side effects [9]. It was found that NaCl addition indirectly causes the precipitation of the drug on the surface of ND, which releases the drug after delivery to the cell [9]. So DOX adsorption on the ND surface and its inverse output can be achieved by adjusting of Cl^- ions [9].

DOX-ND-composites made with adjustable ion concentration of Cl^-, slowly release accumulated DOX, which increases the potential of the composites as therapeutically significant materials [9]. Previous work has shown the significant benefits of NDs which are characterized with the targeted drug delivery to specific sites in the human body that leads to the decrease of the amount of the using drug. So, DOX is accumulated on the ND surface and incorporated between the aggregates of NDs [9].

To determine the adsorption capacity it was studied studied the effect of NaCl on DOX adsorption on the ND surface. When NDs are negatively charged (e.g. ND-COO^-), they interact with DOX cation (DOX-NH_3^+). However, as it was found that DOX + ions are not easily adsorbed on ND- due to the high water solubility of both cations and anions [9]. Therefore, to provide precipitation of ND- and DOX + from aqueous suspension and to create the composite of ND-DOX, NaCl is the necessary component to download DOX on UD surface. Without NaCl, less than 0.5 wt% of DOX is adsorbed on ND. However, adsorption of DOX + on ND- icreased til 10 wt% when NaCl was added (10 mg/ml). Thus, it was shown that increasing of the concentration of Cl– promoted the adsorption of DOX on ND, initiating the formation of ND -DOX + complexes [9].

Compared with other carbon nanomaterials, including C_{60}, carbon nanotubes, and graphene, NDs possess better biocompatibility with different types of cells, including neurons, lungs, kidney and cervix cells [33]. ND can be considered as one of the best biocompatible materials for using in biomedicine than the other carbon nanomaterials. However, despite the positive results obtained with ND, in the way of practical biomedical applications there are a number of problems that should be solved. Like many other nanoparticles, they tend to gather in large clusters in saline through strong interaction between the individual nanoparticles. The plasma proteins adsorbed NDs in human blood [8, 33]. To solve the problems related with ND using in nanomedicine the following methods should be developed:

a) modification and control of the composition of ND surface;

b) improving of dispersion;

c) inhibition of mononuclear phagocyte (MP) action on NDs and adsorption of plasma elements on their surface.

Although recently a number of methods have been developed including surface fluorination, click-chemistry, surface chemical oxidation, laser irradiation, surface initiated polymerization with or without dispersants to improve ND dispersion of [8], only some of them studied the dispersion of ND in saline [8, 9, 15].

However, agglomeration of individual ND particles [15] leads to poor dispersion in physiological environments (eg, in PBS) or in the environment of cell culture that adversely affects the ND using in nanomedicine.

So, in recent years to improve ND dispersion the research has been carrying out to modify their surface with various polymers [15]. As protecting groups for improving ND dispersion the following polymeric systems were used: PEG, polyvinyl alcohol (PVA), polysaccharides, etc. [33]. PEG successfully integrated with various nanoparticles, including quantum dots and nanoparticles of gold. PEG was also used for coating of monodisperse nanoparticles with Fe_3O_4. These particles had little aggregation under conditions of cell culture [78].

Modification of nanoparticle surface with PEG

ND creation implies modification of nanomaterials with synthetic polymer that enhances the stability of nanomaterials and the formation of complexes which are well dispersed in water and can be used in biomedicine. PEG is a hydrophilic polymer with important physical, chemical and biological properties. It is characterized with good water solubility and biocompatibility [8, 15, 33]. There are several variants of surface modification with PEG using polymers with different characteristics. As a rule, researchers used polymers with molecules of different lengths and additional side groups that are used for conjugation of the polymer with the surface of ND or the creation of additional volume ramifications [8]. One method of coating diamond particles with PEG is shown at Fig. 11.

NDs conjugated with poly(ethylene glycol) methylethermetacrylate (PEGMA) possess high dispersibility in aqueous and organic media (correspondingly ND-OH and ND-COOH). Besides, ND-PEGMA have better dispersibility in phosphate buffer solution compared to unmodified ND and possess higher stability compared to ND-OH [8]. ND-PEGMA is characterized with greater dispersion in water, so they are able to create functional nanoparticles of ND for using in biomedicine [33]. Improved dispersion of NDs in organic media may be important for including them as nanofiller in the organic polymer matrix to form nanocomposites [33].

Stability and high dispersion of ND-PEGMA in a physiological environment were likely mediated by hydrophilic PEGMA chains attached to the surface of ND. Ions in phosphate-saline can reduce electrostatic repulsion of ND-OH, which leads to the precipitation of ND-OH in the buffer. Therefore, for applications in biomedicine ND-PEGMA are better candidates because

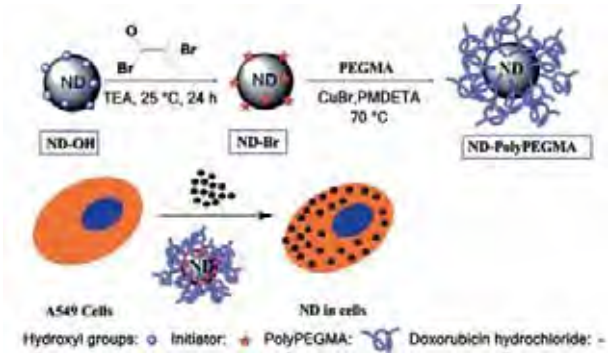

Fig. 11. **Schematic representation for the preparation of poly-PEGMA coated ND by atom transfer radical polymerization (ATRP). Intracellular delivery of doxorubicin via polyPEGylated ND nanoparticles:**
ND-Br was used as the initiator, poly(ethylene glycol)methylethermetacrylate (PEGMA475,) — as monomer and Cu (Br) / N, N, N′, N″, N″-pentamethyl diethylenetriamine (PMDETA) — as the catalyst/ligand [8]

of their high dispersion in phosphate buffer [8]. After intravenous administration of ND are recognized by MP cells as a foreign object and quickly removed from the bloodstream. Due to the cumulative negative impact of nanomaterials on MP-cells, NDs impair the functioning of this vital defense system [8, 38]. PEG is one of the hydrophilic polymers which are used to provide steric and hydrodynamic barrier on the surface of nanoparticles that prevents adsorption of blood plasma proteins [33]. Conjugated PEG-chains on the surface of nanoparticles (liposomes, quantum dots, nanoparticles of gold, carbon nanotubes and other materials) promote essential dispersibility of ND in aqueous media and enhance penetration and stability of nanoparticles. PEG prevents hydrophobic and electrostatic interactions that decreases the protein opsonization of nanoparticles and provides possibility to avoid their quick recognition by the immune system and as a result to increase their residence time in the blood stream [8, 15, 33].

ND are able to bind, transfer and release drugs [15]. To create the composite which can targetly transfer drug, ND-PEGMA was loaded with DOX. After the inclusion of ND-PEGMA in the composite for 48 hours the efficiency of loading with drug can reach 65% (w/w). Compared with the loading, the DOX release from the complex ND-PEGMA is sufficiently slow [8].

After incubation with nanoparticles of PEGMA and ND-OH in the range from 10 to 160 μg / ml for 24 h there were not significant changes in the morphology of A549 cells compared with control. It was established that the viability of A549 cells gradually decreased with increasing concentration of ND-PEGMA-DOX. The significant cytotoxicity was observed at the concentration of ND-PEGMA-DOX 160 μg/ml. The method of confocal laser scanning microscopy showed that the complex ND-PEGMA-DOX entered the cytoplasm and DOX entered the cell nucleus [8]. The obtained results indicate a potential use of PEG-coated ND particles for the controlled drug delivery, gene therapy and tissue engineering [8]. ND after internalization does not cause any cytotoxic or negative effects on the proliferation and differentiation of cells. Acceptable biocompatibility is very important for the application of nanoparticles. It is known that nano-SiO_2 can cause liver damage after intraperitoneal continuous administration of the drug [15]. Taking into consideration the good biocompatibility of nanoparticles ND-PEGMA and their high efficiency for loading drugs they may be promising drug delivery system for therapy [8]. In order to increase dispersion and

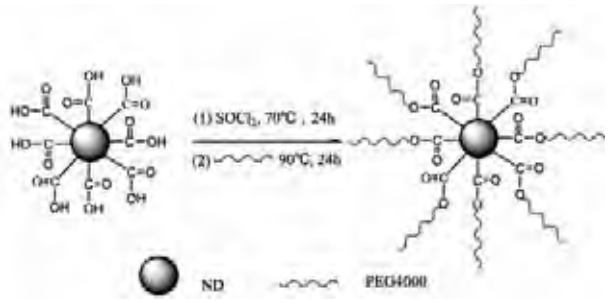

Fig. 12. **Schematic of ND functionalized with PEG-4000 [15]**

stability of ND in physiological environment or cell culture medium, the biocompatible poymer hydroxypolyethylene-4000 (PEG-4000) was covalently conjugated with the surface of carboxylated NDs [15]. The formed complex ND-PEG-OH was tested as a carrier of DOX [15; Fig. 12].

This PEG-4000 was covalently conjugated with NDs using thionyl chloride ($SOCl_2$) and triethylamine [$(C_2H_5)_3N$)]. Modified ND-PEG-4000 in phosphate-buffered saline (pH 7.4) had greater polydispersity than the original ND. Then DOX were adsorbed on ND-PEG-OH particles. The experiments showed that DOX released from composites ND-PEG-DOX slowly. In this study was also found that the dissociation of DOX from ND-PEG-DOX composite has a plateau for about 20 hours, and cumulative release rate was 46.8% . DOX released from ND-PEG-DOX system had a slow and sustained drug releasecapability. ND-PEG-DOX nanoparticles were bioactive and more efficiently released drug compared to ND-DOX. Half-life of ND-PEG-DOX approximately two times higher than ND-PEG-OH absorption of free DOX, which leads to the gradual release of DOX complex in solution. It is known that after intravenous injection of raw diamonds, nanoparticles are mainly accumulated in the lungs and liver [8]. When the influence of ND nanoparticles on A549 (lung carcinoma cell line human) was studied by microscopy it was shown a significant number of ND aggregates on the cell surface [8]. Experimental study of single particles revealed that most of the internalized unmodified FND (size ~35 nm) was within the endosome and could not move freely in the cytoplasm [8]. Results of investigations with confocal fluorescence microscopy showed that the FND-PEG-DOX composites effectively enter the cytoplasm of HepG2 via clathrin-mediated endocytosis (they used clathrin-dependent way) [15]. DOX is separated from the ND-PEG-DOX-composites within the cytoplasm and migrates into nucleus

independently, providing apoptotic effect on tumor cells of human liver HepG2. The rate of absorption of ND-PEG-DOX approximately two times greater compared with free DOX. ND-PEG-DOX complex can act as effective drug carrier. Thus, ND combination with chemotherapy drugs is a promising method, which combines biocompatibility and therapy effectiveness under conditions of overcoming tumor resistance towards treatment. NDs demonstrate the significant potential for the creation of nanosized medical technology to obtain the desired functionalization [9].

Using carbon nanomaterials it was designed and synthesized new nanostructures with high specificity, low toxicity and biocompatibility. Compared to carbon nanomaterials, NDs possess better biocompatibility for different cell types and are considered as the best objects for using in biomedicine.

Thus, the current direction of nanomedicine is to create comprehensive biocompatible nanomaterials with antitumor activity that facilitate drug delivery in a targeted localization of pathological processes and enhance the antitumor activity in combination with traditional chemotherapy, reducing the toxic effects in the body. C_{60}-fullerenes, nanoporous silica, carbon nanotubes and ND are important for target drug delivery.

Encapsulation of drugs to nanocarriers that selectively aimed at malignant cells and application of different drug combinations mitigate the side effects of traditional chemotherapy. Target nanocarriers must simultaneously have specificity, stability and high capacity for conjugation and delivery of various compounds. The complex of nanoporous core having a large surface with the lipid bilayer possesses these qualities. This approach makes it possible to create a universal method of nanotherapy. To improve efficiency of drug delivery researchers use ligands that specifically interact with receptors on the cell surface. Such hybrid nanoparticles loaded with combinations of therapeutic and diagnostic agents, have a greater affinity for tumor cells versus normal. Modification of nanocarrier surface with hydrophilic polymers, including PEG increases the circulation in blood by slowing down the phagocyte absorption.

Recently ND have been considered as a multifunction platform due to cheap industrial detonation synthesis of UD. It was developed the technique for creating of different hydrophilic types of ND surface and for the control of the purity of this surface. This gives broad opportunities for good surface functionalization with organic compounds, including immobilization of biomolecules. Modern methods of refining UD allow create the objects with identical properties obtained from different suppliers.

PEG surface modification provide the nanoparticle with good dispersion properties in aqueous solutions, increases the permeability and stability of nanoparticles, and at the same time prevents the rapid recognition by the immune system, which prolongs the time of their circulation in the body. Fusion of liposomes with a large surface of nanodiamonds followed by modification of the lipid bilayer with multiple copies of target peptides or fusion peptides and covering the whole carrier structure with PEG is the way to create a new type of stable and selective carriers. Such multicomponent hybrid nanoparticles serve as the basis for the controlled release of multicomponent mixtures in sufficient concentration in the cytosol of the tumor cell due to the high specificity, high degree of loading and stability, which is necessary to transport chemically different therapeutical remedies into tumor cells. Besides, they can provide minimal non-specific binding to healthy cells.

The advantage of using ND is to create new technology for drug delivery, which combines the efficiency of transportation with the necessary degree of biocompatibility. ND combination with other nanoparticles, including C_{60}, carbon nanotubes and silica nanoparticles will serve as the basis for a new type of therapy that is promising in the treatment of many diseases, including cancer, cardiovascular, diabetes and others. Such combined nanostructures connected with medical drugs, promote the creation of new biocompatible methods to overcome tumor resistance to the action of drugs and improve the effectiveness of therapy.

Thus, ultrafine fluorescent diamonds are promising materials for using in biomedicine as stable biomarkers. Results of studies using confocal fluorescence microscopy showed that the FND-PEG-DOX composites effectively enter the cytoplasm of target cells via clathrin-mediated endocytosis. DOX is separated from ND-PEG-DOX composites within the cytoplasm and migrates into nucleus independently, providing apoptotic effect on cancer cells. ND have significant potential to create nanosized medical biotechnology with the possibility of broader appropriate functionalization. However, despite the promising results obtained with ND, from creation of advanced materials to their biomedical practical application, there is a number of the mentioned problems that have to be solved in the near future.

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ВИКОРИСТАННЯ НАНОДІАМАНТІВ У БІОМЕДИЦИНІ

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Метою роботи було узагальнення даних літератури стосовно перспектив використання нанодіамантів для створення методів ефективної терапії та доставлення лікарських препаратів. Показано, що розроблені методи доочищення, контролю чистоти поверхні та створення різних гідрофільних типів поверхні нанодіамантів надають широкі можливості для якісної функціоналізації поверхні органічними сполуками.

Сучасним напрямом наномедицини є створення комплексних біосумісних наноматеріалів, які сприяють цілеспрямованому доставленню ліків у місця локалізації патологічних процесів. Для транспортування лікарських препаратів до органів-мішеней важливе значення мають C_{60}-фулерени, нанопористий діоксид кремнію, карбонові нанотрубки, нанодіаманти. Вони мають бути біосумісними, стабільними, з підвищеною дисперсністю у біологічних середовищах, а також не одразу розпізнаватися імунною системою. Такі композити ефективно потрапляють в цитоплазму клітин за допомогою клатринопосередкованого ендоцитозу.

Ключові слова: нанодіаманти, гібридні наноносії, біомедицина.

ИСПОЛЬЗОВАНИЕ НАНОАЛМАЗОВ В БИОМЕДИЦИНЕ

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Целью работы было обобщенние данных литературы относительно перспектив использования наноалмазов для создания методов эффективной терапии и доставки лекарственных препаратов. Показано, что разработанные методы доочистки, контроля чистоты поверхности и создания различных гидрофильных типов поверхности наноалмазов предоставляют широкие возможности для качественной функционализации поверхности органическими соединениями.

Современным направлением наномедицины является создание комплексных биосовместимых наноматериалов, которые способствуют целенаправленной доставке лекарств в места локализации патологических процессов. Для транспортировки лекарственных препаратов к органам-мишеням важное значение имеют C_{60}-фуллерены, нанопористий диоксид кремния, карбоновые нанотрубки, наноалмазы. Они должны иметь хорошую биосовместимость, стабильность и повышенную дисперсность в физиологических средах, а также избегать быстрого распознавания иммунной системой. Такие композиты эффективно попадают в цитоплазму клеток с помощью клатринопосредованного эндоцитоза.

Ключевые слова: наноалмазы, гибридные наноносители, биомедицина.