ISSN 1070-3632, Russian Journal of General Chemistry, 2014, Vol. 84, No. 2, pp. 379–390. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © R.Yu. Yakovlev, A.S. Solomatin, N.B. Leonidov, I.I. Kulakova, G.V. Lisichkin, 2012, published in Rossiiskii Khimicheskii Zhurnal, 2012, Vol. 56, Nos. 3–4, pp. 114–125.

Detonation Diamond—A Perspective Carrier for Drug Delivery Systems

R. Yu. Yakovlev, A. S. Solomatin, N. B. Leonidov, I. I. Kulakova, and G. V. Lisichkin

Lomonosov Moscow State University, Moscow, 119991 Russia
e-mail: yarules@yandex.ru

Received September 1, 2012

Abstract—Analysis of published and author's own experimental data provides convincing evidence for the theoretical possibility to create drug delivery systems on the basis of detonation nanodiamonds and for the promise such systems hold for commercialization and practical application.

DOI: 10.1134/S1070363214020406

INTRODUCTION

Development of drug delivery systems is one of the central problems of modern nanochemistry, nanomedicine, and biopharmacy. An important aspect of this problem is search for drug carriers [1, 2]. The most common drug carriers are liposomes, micelles, biodegradable polymers, serum albumin, and polysaccharides [3]. Over the past years, different forms of nanocarbons, specifically, fullerenes and nanotubes, have become considered as potential drug carriers [1–6]. In particular, some examples of the use of nanocarbons in photodynamic therapy for HIV-1 protease inhibition and as nanocontainers for delivery of drugs and diagnostic agents have been reported [7, 8]. Just these works have drawn researcher's attention to another allotropic form of carbon–diamond.

Nanodiamond produced by detonation synthesis is considered as a perspective drug nanocarrier in view of their structural and surface properties, as well as manufacturing parameters [9–12]. Detonation nanodiamond is manufactured from explosives subject to utilization, which makes its commercialization economically efficient. The cost of detonation nanodiamond is $5–10 per gram, its industrial production (tons per year) has been already organized, and market potential is constantly extending.

Nanodiamond has found application in, in particular, the manufacture of polishing materials and metal–diamong galvanic coatings, as well as as an additive for motor oils, lubricants, polymer compositions, and rubbers [13]. Attempted use of nanodiamond in biomedicine date back to 1995, when Kossovsky et al. tried diamond nanoparticles as antigen delivery vehicles [14]. Extensive biomedical research of nanodiamond was initiated in 2000, and, therewith, nanodiamond in itself was considered as a biologically active substance. As shown in [15, 16], nanodiamond suspensions relieve the state of oncological patients, probably, by decreasing the level of intoxication. Furthermore, it was used to correct protein and lipid peroxide oxidation processes associated with malignant tumor growth.

The interest in nanodiamond as a carrier for biologically active substances, including drugs, and, what is more, a material exhibiting intrinsic biological activity, is constantly growing. The proposed applications of nanodiamonds include biomarkers, biosensors, high-efficiency adsorbents, coatings for surgical instruments, cosmetic compositions, UV-screening creams, and additives for dental materials [17].

The aim of the present work is to summarize the available literature and our own data on the biopharmaceutical properties of detonation nanodiamond, consider the chemical approaches to drug delivery systems on its basis, and to provide evidence for a high promise detonation nanodiamond holds for biomedicine.

Detonation Nanodiamond

Synthesis and Purification

Detonation nanodiamond is manufactured either from individual explosives with a negative oxygen balance (triton, hexogen, octogen) or from their

Table 1. Characteristics of selected commercial detonation nanodiamonds

Characteristics	Producer and Trademark		
	Adamas Nanotechnologies (USA), Standart ND	PlasmaChem (Germany), PL-D-G01	Tekhnolog FGUP SKTB (Russia), UDA-TAN
S_{sp}, m^2/g	(300–400)[a]	(350—390)[a]	223±13
Size of nanodiamond aggregates[b], nm	555 200[a]	550 600[a]	50
Ash content, wt %	1.2–1.6[a]	<1.4[a]	0.85[a]
Elemental composition of the surface[c], at %	C 90.2 O 8.5 N 1.3	C 86.2 O 12.2 N 1.6	C 91.3 O 8.7 N 1.0

[a] Producer's data. [b] In water, after ultrasonication. [c] X-ray photoelectron microscopy data; normed for 3 elements.

mixtures [18]. The product formed by detonation is a diamond-containing condensed carbon (diamond charge) which, along with a nondiamond (easily oxidized) carbon, contains a diamond carbon whose content, according to [19], can reach 75 wt %.

Condensed carbon is subjected to gas-phase [20] or liquid-phase oxidation to isolate nanodiamond and prepare it for use [21]. As this takes place, nondiamond structures that are more sensitive to oxidation gradually decompose. The most efficient reagents for liquid-phase oxidation are mixtures of acids ($HClO_4$, H_3PO_4, H_2SO_4), active oxidants (HNO_3, nitrogen oxides, H_2O_2, $NaClO_4$, CrO_3, $K_2Cr_2O_7$, etc.), as well as dilute nitric acid which reacts under pressure (100 atm) at 240–260°C [22]. The latter methods are environmentally the most friendly, and yields nanodiamond with a purity of higher than 99 wt %.

Oxidation of diamond charge forms on the surface of nanodiamond particles a layer containing a wide variety of functional groups. Just this layer is responsible for the existence of a great diversity of nanodiamond modifications [23]. Depending of the composition of the starting explosives, detonation conditions, and, first of all, purification conditions, nanodiamonds have different surface chemical compositions and admixture contents. At present there are two trademarks of nanodiamond, with radically different chemical and physicochemical characteristics, and, therewith, producers not infrequently characterize commercial products by different different parameters. Moreover, nanodiamonds from different batches of the same trademark, too, may differ from each other. As a result, the resulting materials are not equivalent to each other, and this fact should be borne in mind, when they are used in biomedicine, especially as drug delivery vehicles [24].

Structure and Properties of Nanodiamond

Nanodiamond has a unique set of physicochemical characteristics, such as small particle size (4–6 nm), highly developed specific surface, and high concentration of surface functional groups. Nanodiamond particles comprise a diamond core and a perturbed carbon shell having surface functional groups [25, 26]. Just such structural features set nanodiamond apart from carbon nanotubes and fullerenes which are hardly amenable for chemical modification.

The fraction of surface carbon atoms in the total number of atoms in a 4.2-nm nanodiamond particles is ~15% [27]. Therefore, many characteristics of nanodiamond are strongly dependent on its surface characteristics [25].

The structure of the perturbed carbon shell and the composition of surface functional groups in commercial nanodiamonds from different producers differ from each other and depend on synthesis and purification conditions (Table 1). The chemical and physicochemical parameters of nanodiamond are unified by means of surface chemical modification.

Chemical Modification of Nanodiamond Surface

The principal chemical processes allowing targeted modification of the functional composition of the nanodiamond surface, include oxidation, reduction, halogenation, and amination reactions [28, 29]. Oxidative modification of nanodiamond surface is generally performed using oxidant acids (HNO_3, $HClO_4$, etc.) or air oxygen under heating [13]. Liquid-

phase oxidation forms carboxy groups on the on nanodiamond surface, which allows further modification. Reductive modification of nanodiamond is used for generation of hydroxy groups which, too, serve as functional centers for covalent binding. Usually they are formed by treatment of oxidized nanodiamond by strong reducers–boranes (as a complex with THF, $LiAlH_4$, $NaBH_4$ [30]). These reactions allow the surface carbonyl groups to be reduced to hydroxyls. Nanodiamonds with surface amino groups are produced from preliminarily hydrogenated or halogenated nanodiamond by exposure of the latter to gaseous ammonia [30]. Halogenation of hydrogenated nanodiamond is applied to activate the surface immediately before its covalent modification. Gaseous fluorine [31] or CF_4 or SF_6 plasma [32, 33] are used for fluorination. Chlorination of nanodiamond surface is performed by thermo- or photoinitiation of molecular chlorine [34, 35], as well as by means of chlorinating agents, in particular, thionyl chloride [36] or CCl_4 plasma.

Fluorinated nanodiamonds represent materials with new physicochemical properties, they are used in industry as final products [37]. There are a number of reasons why we consider it inexpedient to use fluorinated nanodiamonds as intermediate products in the development of drug systems. First, the C–F bond (E_b = 115 kcal/g-atom) is stronger than the C–Cl bond (E_b = 78 kcal/g-atom), and fluorocarbons are inert to a great number of substances. Therefore, it can be expected that the substitution of fluorine atoms by drug molecules will prove much more difficult to accomplish and require harder conditions to occur, which is unacceptable with many drug substances. We showed that complete substitution of fluorines by drug molecules is not always the case, and, therefore, the resulting conjugates contain admixtures with unsubstituted fluorine atoms. As known, substances with unsubstituted fluorine atoms exhibit enhanced toxicity and may have a negative impact on the nervous system, lungs, and liver [38]. Even perfluorinated compounds, even though being chemically inert, affect parameters of hepatic microsomal xenobiotic-metabolizing [38]. Thus, fluorination of fullerene enhances its general toxicity by a factor of 2.4–5 [39]. By contrast, in chlorinated nanodiamonds, an almost complete substitution of chlorines by grafted molecules is possible, thus avoiding uncontrolled toxicity enhancement in the product. This is extremely important for drugs and medical products. In our work, we modified the nanodiamond surface by liquid-phase chlorination with a solution of molecular chlorine in CCl_4 under visible-light irradiation [33].

Annealing and hydrogenation of nanodiamond can be considered as a separate group of reactions. Annealing in an inert atmosphere at temperatures above 800°C results in surface graphitization of nanodiamond particles, which, according to [40], can be used for further modification. However, we suggest that even a low content of functional groups on the graphitized diamond surface cause its inactivation. Hydrogenated nanodiamond (annealed under hydrogen) has bifunctional surface groups (C_{diam}–H and C_{diam}–OH) [26]. Hyrdogenation not only allows unification of the surface of nanodiamond, but also favors its purification and deaggregation. Thus we showed that the hydrogenation of nanodiamond (800°C, 5 h) makes it possible to reduce the average size of aggregates in hydrosol and enhances the sedimentation stability of the latter [41].

There is a possibility to obtain hydrosols with a high content of hydrogenated nanodiamond (up to 50 g/L). They are stable for 6 months, unlike hydrosols of the parent nanodiamond, in which particle sedimentation is observed already within 30 min even after ultrasonic treatment.

Development of Drug Delivery Systems on the Basis of Detonation Nanodiamond

Nanodiamond Deaggregation

When developing drug delivery systems, one has to deal with the problem of aggregation of primary nanodiamond particles and stability of their sols in aqueous and organic media. Our experiments showed that 75–80 wt % of primary aggregated nanodiamond particles of different brands can be converted into nanoparticles 10–20 nm in size exclusively by chemical modification of their surface followed by ultrasonic treatment. The rest 15–20% of particles seem to be quite strong aggregates, and to destroy them requires additional treatment.

Nanodiamond Drug Delivery Systems

Presently it has become obvious that surface chemical modification is a key tool to impart diverse physicochemical, biopharmaceutical, and biological properties to nanodiamond and to develop on its basis efficient drug delivery systems. Therewith, drug–carrier binding involves exclusively the surface of nanodiamond and does not involve its core.

Nanodiamond drug delivery systems can be obtained in two ways: by adsorption of a drug on the surface of nanodiamond and by covalent binding of a drug with surface functional groups [42]. Scarce information is available on comparative efficiency of these two approaches.

Adsorption of Drugs on the Surface of Nanodiamond

The adsorption technology of the preparation of drug delivery systems on the basis of nanodiamond features facile determination of drug concentration (UV–Vis spectrophotometry). The concentration of the active component can be varied over a wide range, and drug release of the nanodiamond surface can be easily controlled by varying the pH or ionic strength of the solution.

Nanodiamond, in view of its high adsorption capacity, is considered as a promising adsorbent for proteins and enzymes. The results of research on the adsorption of cytochrome C and tripsin on a carboxylated nanodiamond surface [43, 44] and of lysocim on an oxidized surface [45] showed that the adsorption increases with decreasing size of nanodiamond particles. Thus, the adsorption of lysocim on 5-nm particles is 3 times that on 100-nm particles. Therewith, the activity of lysocim on 5- and 100-nm particles comprises 18 and 77% of the activity of the free enzyme, respectively. Consequently, by varying the size of nanodiamond particles one can purposefully vary the activity of adsorbed substances.

A modified nanodiamond surface (oxidized or reduced) allows control of the adsorption process [46]. Therewith, the chemical nature of adsorbates and the structural and volume characteristics of their molecules have a great impact on adsorption. For example, Yeap et al. [47] have studied the effect of steric factors on the adsorption of glycoproteins on the nanodiamond surface and proposed to use a grafted layer of linker chains to ensure surface–adsorbate binding. The adsorption of glycoproteins (fetuin, ovalbumin, etc.) on the nanodiamond surface with a linker layer was found to be 4 times more efficient than on a hydroxylated nanodiamond, where only nonspecific adsorption takes place.

Further evidence for the effect of steric factors on the adsorption of biologically active substances on the nanodiamond surface was obtained by Purtov et al. [48], who found that modified nanodiamonds adsorb linear DNA molecules and do not adsorb ring-shaped ones. At the same time, Grichko et al. [49] observed adsorption of a supercoiled pUC18 plasmid DNA on the nanodiamond surface. This controversy is probably explained by the fact that the nanodiamonds used in the cited works were not standardized or had different functional compositions (which is virtually the same).

A series of works focused on the adsorption on the nanodiamond surface of socially significant drugs: anticancer (doxorubicin) [50–53], antidiabetic (insulin) [54], and antitubercular (isoniazid [55] and amikacin [29]). Developers of nanodiamond delivery systems for such drugs have the aim to improve their permeation into body cells and tissues and reduce their doses and toxicity. An attempt was undertaken to develop a transdermal formulation of doxorubicin [51].

An important direction of these works was research into alteration of the specific activity of an adsorbed drug on the nanodiamond surface. Krueger and Waag [54] showed that adsorbed insulin has a reduced activity, and its activity is restored only when it has desorbed from the nanodiamond surface. At the same time, doxorubicin adsorbed on a hydroxylated and/or carboxylated nanodiamond exhibited reduced toxicity and enhanced specific activity in vivo [52].

Chen et al. [56] studied adsorption of such clinically significant pharmacopeial drug substances as purvalanol A (liver cancer drug), 4-hydroxytamoxifen (breast cancer drug), and dexamethasone (broad-spectrum anti-inflammatory drug). A conclusion was drawn that the use of nanodiamond as a vehicle for drug delivery not only solves the problem of water-insoluble drugs, but also improves their targeting, thus making it possible to reduce the therapeutic dose and toxicity of the drugs.

We used the example of an amikacin antibiotic to show that the adsorption capacity of the nanodiamond surface is much dependent on the nature of surface functional groups [57]. Thus, the maximum equilibrium adsorption of amikacin (c 30 mg/mL) on carboxylated and hydrogenated nanodiamonds was found to be 130 and 50 mg/g, respectively. A high stability of the resulting systems was evidenced by the fact that in a month up to 80 and 71% of amikacin still remained on the surface of carboxylated and hydrogenated nanodiamonds [57].

Drug Grafting on the Surface of Nanodiamond

Nanodiamond as a carrier in drug delivery systems offers an essential advantage: The presence of different functional groups on its surface allows selective

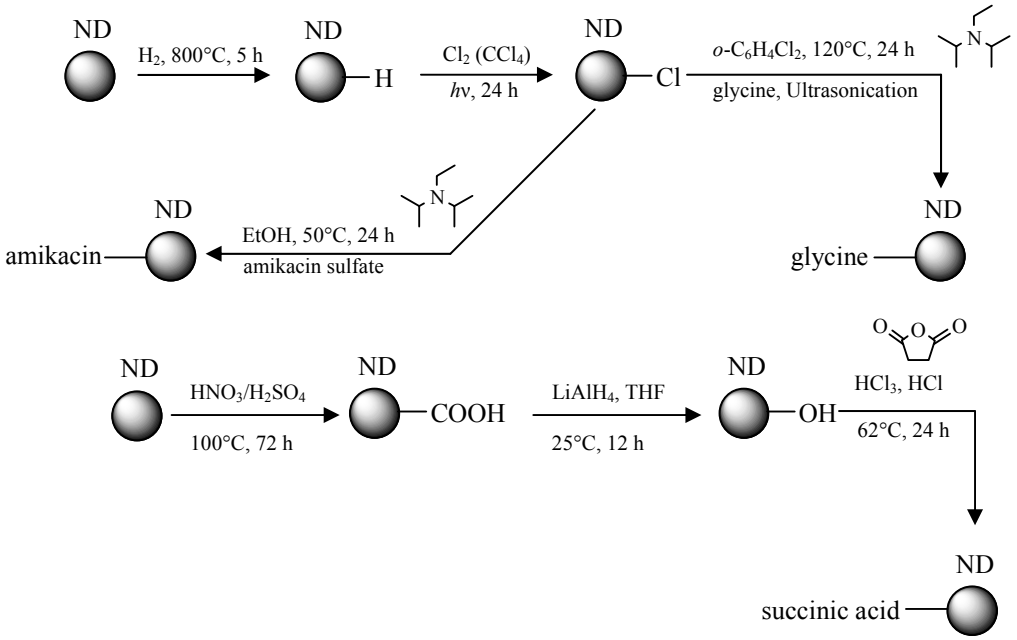

Fig. 1. Schemes of the synthesis of nanodiamond (ND) conjugates with amikacin, glycine, and succinic acid.

covalent binding of drugs. One can choose a modified nanodiamond containing specific surface functional groups capable of binding one or another drug. The covalent binding of drugs with the nanodiamond surface ensures their prolonged action due to accumulation of drug-loaded nanoparticles in target body's organs and tissues. Together with the safety and biocompatibility of nanodiamond carriers [58, 59], this makes it possible to enhance the efficiency of therapy of severe diseases (cancer, tuberculosis, syphilis, etc.) even with known drugs, when an issue of key importance is to reduce their therapeutic dose but preserve efficiency [60]. There are also patent applications (in the USA) for nanodiamond as a carrier capable of enhancing efficiency of certain drugs [61, 62]. Techniques of covalent modification of nanodiamond and linker molecules for binding drug substances are also being patented [63].

The covalent binding with the nanodiamond surface of DNA [64], glycine [31], bovine serum albumin [65], biotin [66], trypsin and asparagines F [67], ethylenediamine [68], sugars [69], dopamine derivatives [70], pactitaxel [71], and others has been reported. It is very important that conjugated drugs preserved their specific activity. As found in a series of works on the covalent binding of enzymes to the nanodiamond surface, the enzymatic activity changed only slightly [67]. Moreover, the covalently bound enzymes exhibited enhanced thermal and chemical stability and retained high activity even after 10 uses [67]. Liu et al. [71] showed in their cell viability inhibition study that the anticancer drug paclitaxel covalently bound via a linker chain fully retained its specific activity [71].

We prepared nanodiamond delivery systems for glycine (central neuromediator), amikacin (antibiotic used for tuberculosis), and cinnamic acid (Krebs cycle intermediate) (Fig. 1).

The nanodiamond conjugates with glycine and amikacin were synthesized from a chlorinated nanodiamond by substitution of its chlorine atoms by the amino groups of the drugs in the presence of *N,N*-diisopropylethylamine. Therewith, of the four amino groups of amikacin (Fig. 2) the reaction was probably involved the most sterically accessible group of the 2-hydroxy-1-oxobutyl fragment of [1.4]-2-deoxy-D-streptamine. By the way, the semisynthetic antibiotic amikacin was obtained in 1972 just by the addition of the 4-amino-2-hydroxy-1-oxobutyl radical to the N1-amino group of kanamycin [72].

The conjugate of nanodiamond with cinnamic acid was prepared by the reaction of a hydroxylated nanodiamond with cinnamic anhydride. The hydroxylated nanodiamond was synthesized in two stages: First nanodiamond was oxidized with a mixture of nitric and sulfuric acid and then the resulting product was treated with $LiAlH_4$ to reduce its carboxy groups to hydroxyls.

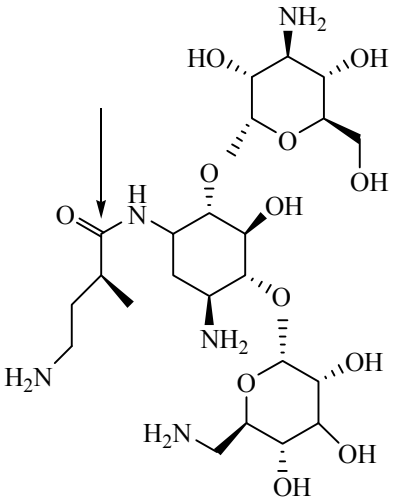

Fig. 2. Structure of amikacin (the arrow shows the site of addition of the 4-amino-2-hydroxy-1-oxobutyl radical to kanamycin).

The chemical state of the nanodiamond surface at all modification stages was traced by IR and X-ray photoelectron spectroscopy.

We developed an IR procedure for estimating the contents of solid-phase nanodiamond–drug conjugates [73], which involves construction of a signal intensity–drug weight calibration function [74]. The contents of conjugated drugs in the glycine–nanodiamond and amikacin–nanodiamond conjugates were estimated at 3 and 0.7 molecules/nm^2, respectively. The succinic acid–nanodiamond conjugate does not give characteristic bands in the IR spectrum, and, therefore, the content of conjugates acid was estimated indirectly by a procedure based on the model of isolated mitochondria in comparison with free succinic acid (under the assumption that succinic acid does not change its activity on binding). The binding density of succinic acid was estimated at 0.2 molecules/nm^2.

According to transmission electron microscopy (TEM) data, the glycine–nanodiamond conjugate particles have a shell (Fig. 3a). Its formation can be associated with a high concentration of glycine molecules bound to the nanodiamond surface, which correlates with the mentioned estimate. At the same time, no shell was observed both in the parent (chlorinated) nanodiamonds and in the conjugates of nanodiamond with amikacin and succinic acid (Figs. 3b and 3c). Biopharmaceutical and biological properties of nanodiamond–drug conjugates

The biological effect of the glycine–nanodiamond and amikacin–nanodiamond conjugates was preliminarily assessed *in vitro* [75]. To this end, HeLa cells were cultured in a nutritive medium with nanodiamond hydrosols for 15 min and 2, 8, and 24 h, embedded in Epon resin, after which ultrathin sections (75 nm) were subjected to TEM examination.

In the TSM images (Fig. 4), on the background of cell structures one can clearly see nanodiamond particles which represent nanocrystals about 5 nm in size. The ultrastructural study showed that in the course of 15 min of incubation nanodiamonds bind to cell membranes and begin penetrate cells. The cell membrane interacts with nanodiamond particles and invaginates them gradually; eventually, the cell encapsulates nanoparticles (Fig. 4a). The 3D-analysis on serial ultrathin sections showed that after 15-min incubation nanodiamond particles had already been entrapped by cells (Fig. 4b). Therewith, the particles are not encapsulated by the cell membrane and freely move in the cytoplasm. This fact provides evidence

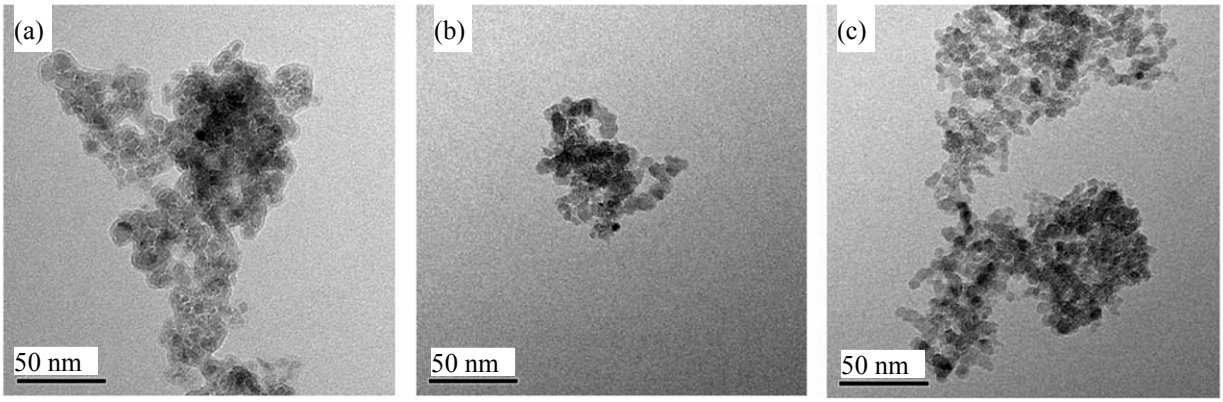

Fig. 3. Micrographs of nanodiamond conjugates with (a) glycines, (b) amikacin, and (c) succinic acid.

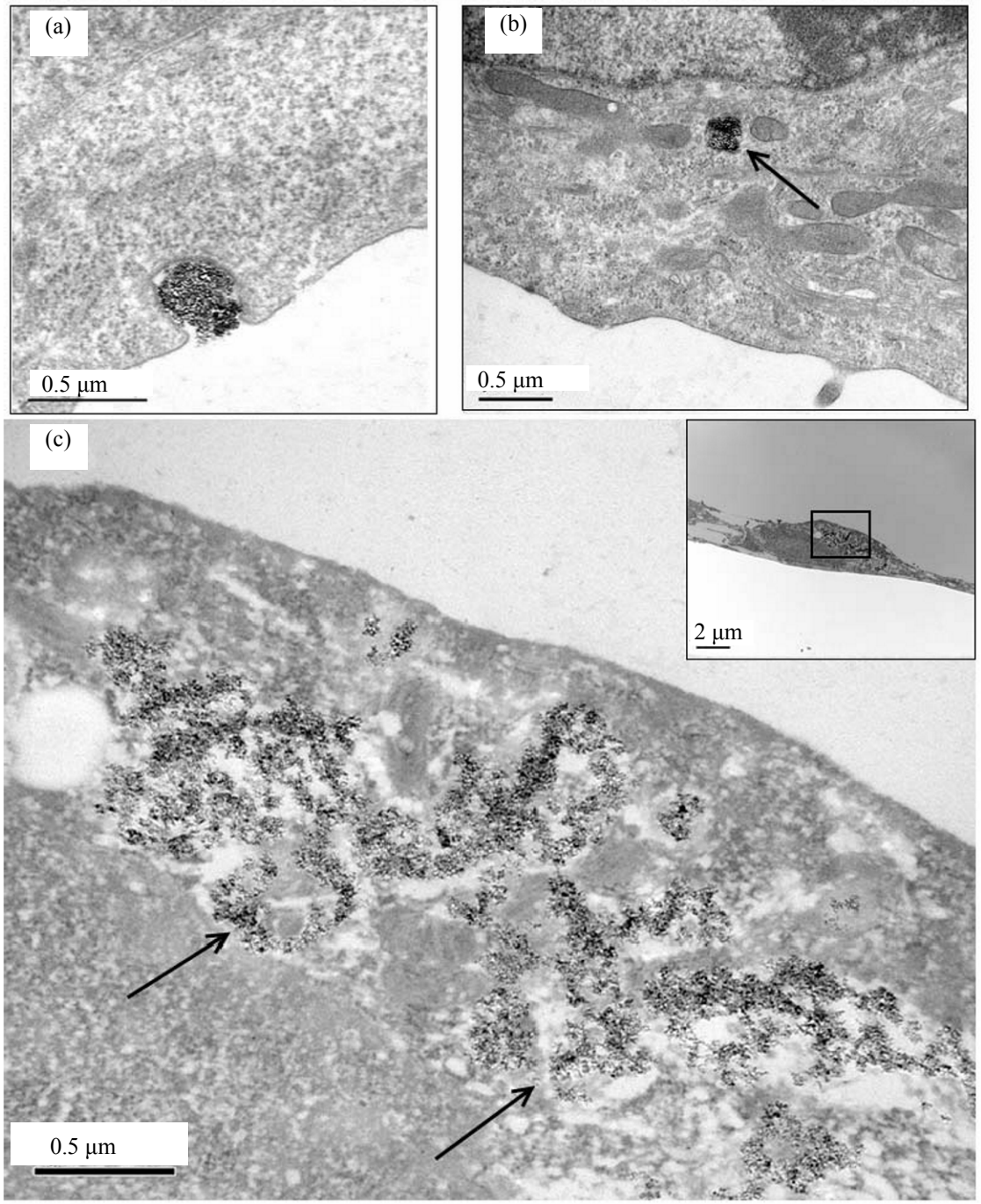

Fig. 4. Micrographs of nanodiamond with HeLa cells: (a) nanodiamond particles permeated the cell membrane via invagination after 15-min incubation, (b) nanodiamond particles permeated the cell after 15-min incubation, and (c) accumulation of nanodiamonds in the cell after 24-h incubation.

showing that nanodiamond particles are nontoxic and biocompatible with living cells. The results of 24-h incubation of the nanodiamond–amikacin conjugate revealed toxicity for HeLa cells. Taking into account that the initial nanodiamond and its conjugate with glycine showed no toxicity effects, the toxic effect can only be related to the amikacin antibiotic. Moreover, it was found that the nanodiamond–amikacin conjugate much more rapidly penetrates cells than the initial nanodiamond (Table 2).

Table 2. Dynamics of HeLa cell permeation of nanodiamond and its conjugates with drugs

Studied substance	Fraction of cells inside the nanodiamond particle (%) at the incubation time			
	15 min	2 h	4 h	24 h
Nanodiamond initial	38.9	85.7	90.2	88.7
Nanodiamond–glycine	2.9	3.9	67.9	70.7
Nanodiamond–amikacin	3.8	44.8	45.3	87.3

The behavior of nanodiamond particles in vivo has still scarcely been studied [76–79]. Thus, the first research on nanodiamond biodistribution Yuan et al. [76] made use of the radioactive label ^{125}I. However, the authors of the mentioned work used not a detonation nanodiamond, but the nanodiamond obtained by crushing coarse synthetic diamond crystals, whose particles with a size of ~50 nm have sharp cleaved edges. Experiments showed that up to 60% of such diamond crystallites injected intravenously in mice were accumulated in liver at 30 min post-injection, and no clearance was observed within 28 days post-injection. These nanodiamonds were also accumulated in spleen and liver. Visual examination of mice revealed no toxic effects of the studied nanodiamonds: The animals did not lose body weight and appetite, and no vomit and diarrhea were observed [76].

Zhang et al. [77] studied the biodistribution of detonation nanodiamonds with a size of 2–8 nm and a purity of higher than 95%, using the ^{188}Re radioactive label, after intratracheal instillation within 48 h. It was found that nanodiamonds were entrapped in the spleen, liver (mostly), bones, and heart. Histological analysis indicated that nanodiamonds could induce a dose-dependent toxicity to the lungs, liver, kidney, and blood. The observed toxic effect can be associated with an insufficient purity of the nanodiamond used in the cited work. Thus, some information is available that the toxicity of carbon materials, in particular, nanotubes, depends on the concentration of their metal impurities [80]. At the same time, histological results in a number of medical research gave evidence for the biocompatibility and nontoxicity of nanodiamond [81, 82]. Tyan [81] studied the chronic effect of nanodiamond hydrosols (particle size 100 nm, concentration up to 0.05 wt %) in mice for 6 months. No morphological changes in mice organs were observed at the total dose of nanodiamonds of up to 0.45 g. The state of all organs, including excretory organs, did not differ from the control group. Lazarenko [82] established that nanodiamond hydrosols not only caused no pathology in intact parodontium tissues of experimental animals, as evidenced by a lack of inflammation both in the injection site and in the parodontium, but also had a stabilizing effect on an inflammatory process in parodontium tissues. Improvements in the mucous membrane of gingivae and significant reduction of the depth of dental pockets were also mentioned.

Yuan et al. [78] studied the toxicity, accumulation, and lung clearance of detonation nanodiamond (particle size 5 nm) and crushed synthetic nanodiamond (particles size 50 nm) on intratracheal instillation in mice. The hystopathological and ultrastructural examinations led the authors to conclude that nanodiamond had no evident negative impact on the lung tissue throughout the entire experiment period (28 days). The clearance of nanodiamonds from the lungs was associated with their absorption by alveolar macrophages followed by consecutive clearance in the trachea and throat.

Qi et al. [79] studied the in vivo biodistribution of nanodiamonds, both individually and together with carbon nanotubes. Nanodiamonds and nanotubes were visualized by means of ^{99}Tc. After a single intravenous injection in mice, nanodiamond preferentially accumulated in the liver, spleen, and lungs. After 24 h, the nanodiamond level in the lungs decreased, while in the liver and spleen continued to increase. The authors explained this observation by the displacement of nanoparticles from the lungs to circulatory system and further consecutively to liver and spleen. The high level of nanodiamond accumulation in organs in explained by the small size of nanoparticles and their rapid capture by the reticuloendothelial system via opsonization and capture by macrophages. The biodistribution of nanodiamond after its co-injection with nanotubes changes radically. Thus, when the mixture contained more nanotubes than nanodiamonds, the hepatic and splenic accumulation of the latter decreased, while their lung retention gradually increased. In their turn, nanodiamonds did not essentially affect the bioaccumulation of nanotubes in vivo.

In our work, to gain insight into the biodistribution and clearance of nanodiamond in animal (rabbit) experiments, we used as a label its covalent conjugate

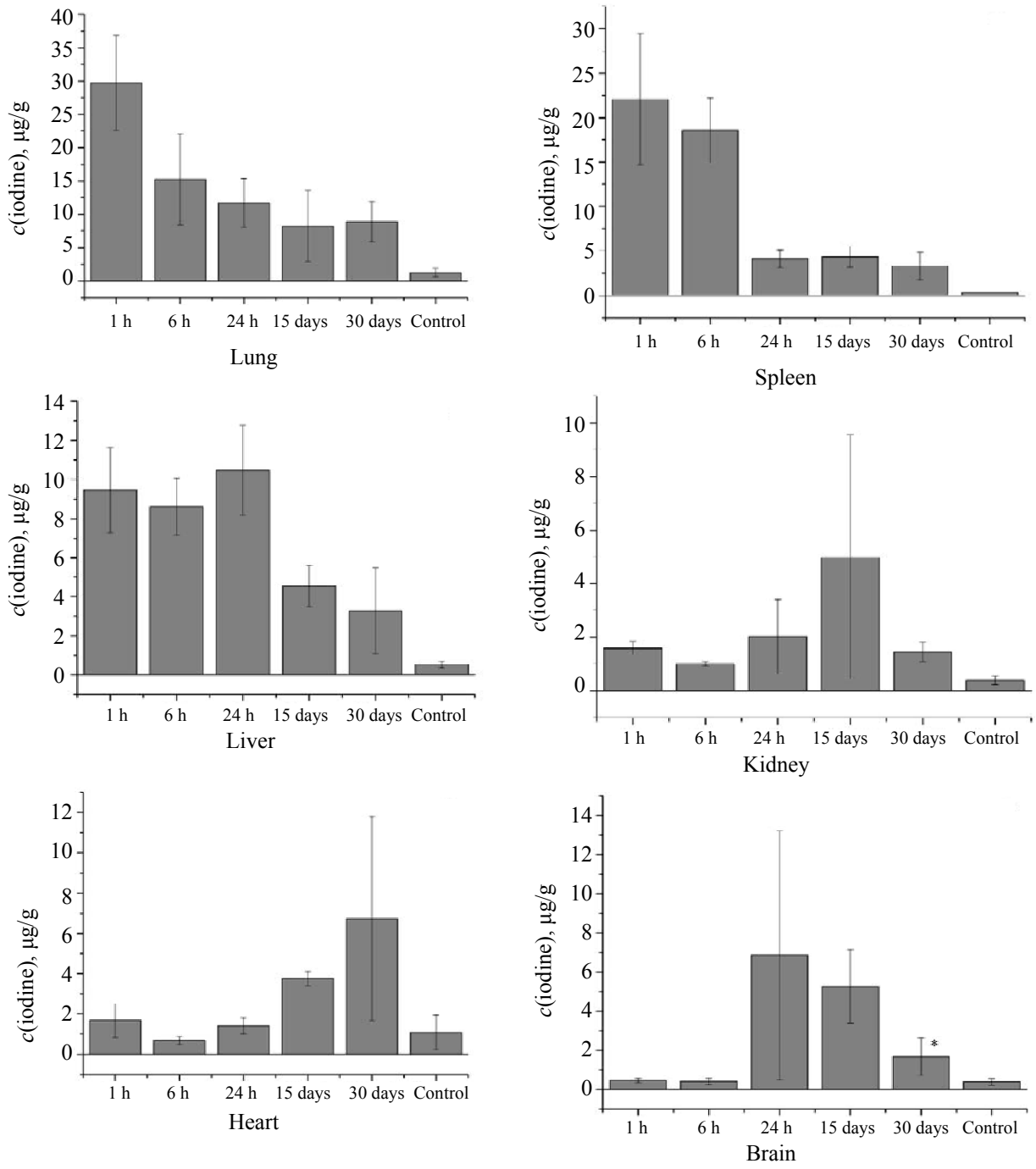

Fig. 5. Dynamics of nanodiamond accumulation in and clearance from rabbit organs (averaged over 5 animals).

of triiodobenzyl alcohol as a single intravenous injection. The iodine accumulation in organs was measured for one month (Fig. 5). It was established that nanodiamond was fairly rapidly (1 h post-injection) and most accumulated in the rabbit lungs (up to 30 μg/g) and spleen (23 μg/g). The limiting accumulation of nanodiamond in other organs occurred slower: within a day in the liver and brain and within 15 days in the kidney and heart. The nanodiamond accumulation in the heart and brain was estimated at 6.5 μg/g. The clearance of nanodiamond from almost all organs occurs within a month (Fig. 5).

ACKNOWLEDGMENTS

The authors express their sincere gratitude to Cand. Phys. Sci., Docent N.G. Seleznev, Cand. Biol. Sci., Senior Researcher N.I. Fedocheva, Doct. Med. Sci., Prof. D.R. Rakita, Doct. Biol. Sci., Prof. R.E. Uzbekov, Doct. Med. Sci., Prof. V.E. Sinitsyn, Doct. Med. Sci., Prof. A.M. Shestakov, Corr. Member RAMS, Doct. Med. Sci., Prof. A.V. Nikolaev, and Doct. Biol. Sci., Prof. M.N. Kondrasheva for support, discussions, and invaluable help in the present work.

The work was financially supported by the Russian Foundation for Basic Research (project no. 11-03-00543).

REFERENCES

1. *Nanotherapeutics: Drug Delivery Concepts in Nanoscience*, Lamprecht, A., Ed., Singapore: Pan Stanford, 2008.
2. Men'shutina, N.V., *Nanochastitsy i nanostrukturirovannye materialy dlya farmatsevtiki* (Nanoparticles and Nanostructured Materials for Pharmaceutics), Kaluga: Izd-vo N.V. Bochkarevoi, 2008.
3. Gupta, R.B. and Kompella, U.B., *Nanoparticles Technology for Drug Delivery*, New York: Taylor & Francis, 2006.
4. Zhu, Y., Li, J., Zhang, Y., Yang, X., Chen, N., et al., *Theranostics,* 2012, vol. 2, no. 3, pp. 302–312.
5. Kroto, H.W., Heath, J.R., O'Brien, S.C., Curl, R.F., and Smalley, R.E., *Nature,* 1985, vol. 318, pp. 162–163.
6. Iijima, S., *Nature,* 1991, vol. 354, pp. 56–58.
7. Friedman, S.H., Decamp, D.L., Sijbesma, R.P., Srdanov, G., et al., *J. Am. Chem. Soc*., 1993, vol. 115, no. 15, pp. 6506–6509.
8. Pantarotto, D., Partidos, C.D., Hoebeke, J., Brown, F., et al., *Chem. Biol*., 2003, vol. 10, pp. 961–966.
9. Leonidov, N.B., Selezenev, N.G., and Yakovlev, R.Yu., *Meditsina v Kuzbasse*, 2009, spets. vyp. 7, pp. 56–57.
10. Shrand, A.M., Hens, S.A.C., and Shenderova, O.A., *Crit. Rev. Solid State Mater. Sci*., 2009, vol. 34, pp. 18–74.
11. El-Say, K.M., *J. Appl. Pharm. Sci*., 2011, vol. 1, no. 6, pp. 29–39.
12. Danilenko, V.V., *Phys. Solid State*, 2004, vol. 46, no. 4, p. 711.
13. Dolmatov, V.Yu., *Ul'tradispersnye almazy detonatsionnogo sinteza* (Ultradisperse Detonation Diamonds), St. Petersburg: Sankt-Peterg. Gos. Ped. Univ., 2003.
14. Kossovsky, N., Gelman, A., Hnatsyszyn, J., Rajguru, S., et al., *Bioconjug. Chem*., 1995, vol. 6, no. 5, pp. 507–511.
15. Dolmatov, V.Yu. and Kostrova, L.N., *Sverkhtverd. Mater*., 2000, no. 3, pp. 82–85.
16. RF Patent no. 2203068, 12.04.2001.
17. Sung, J. and Lin, J., *Diamond Nanotechology: Synthesis and Applications*, Sung, G., Singapure: Pan Stanford, 2010, pp. 163–191.
18. Lyamkin, A.I., Petrov, E.A., Ershov, A.P., Sakovich, G.V., et al., *Dokl. Akad. Nauk SSSR*, 1988, vol. 302, no. 3, p. 611.
19. Dolmatov, V.Yu., *Russ. Chem. Rev*., 2001, vol. 70, no. 7, p. 607.
20. RF Patent no. 2168462, 10.06.2001.
21. Dolmatov, V.Yu., Veretennikova, M.V., Marchukov, V.A., and Sushchev, V.G., *Phys. Solid State*, 2004, vol. 46, no. 4, p. 611.
22. RF Patent no. 2109683, 5.03.1996.
23. Dolmatov, V.Yu., Sushchev, V.G., Fudjimura, T., and Veretennikova, M.V., *Sverkhtver. Mater*., 2004, no. 1, pp. 23–29.
24. Osipova, A.S., Yakovlev, R.Yu., and Kulakova, I.I., Abstracts of Papers, *5-ya Vseross. konf. s mezhdunarodnym uchastiem "Khimiya poverkhnosti i nanotekhnologiya"* (5th All-Russia Conf. with Int. Participation "Surface Chemistry and Nano-technology"), Khilovo, September 23–30, 2012, pp. 246–247.
25. Kulakova, I.I., *Phys. Solid State*, 2004, vol. 46, no. 4, p. 636.
26. Kulakova, I.I., Korol'kov, V.V., Yakovlev, R.Yu., and Lisichkin, G.V., *Ross. Nanotekhnol*., 2010, vol. 5, nos. 7–8, pp. 66–73.
27. Spitsyn, B.V., in *"Inzhenerno-khimicheskaya nauka dlya peredovykh tekhnologii", Mezdunar. shkola povysheniya kvalifikatsii "Problemy i dostizheniya fiziko-khimicheskoi i inzhenernoi nauki v oblasti nanomaterialov"* (Collected Papers "Engineering Chemical Science for Abvanced Technologes," Int. Qualification Course "Problems and Advances of Physicochemical and Engineering Science in the Field of Nanotechnology"), 2002, series 7, pp. 101–123.
28. Krueger, A. and Lang, D., *Adv. Funct. Mater*., 2012, vol. 22, pp. 890–906.
29. Yakovlev, R.Yu. and Leonidov, N.B., *Int. Conf. "Diamond and Carbon Materials",* Granada, September 3–6, 2012, p. 2.6. *https://elsevier.-conference-services.net/secureProgramme.asp?conferenceID=3113.*
30. Krueger, A., *Carbon Materials and Nanotechnology*, Weinheim: Wiley–VCH, 2010, pp. 329–388.
31. Liu, Y., Gu, Z., Margrave, J.L., and Khabashesku, V.N., *Chem. Mater*., 2004, vol. 16, no. 20, pp. 3924–3930.
32. Scruggs, B.E. and Gleason, K.K., *J. Phys. Chem*., 1993, vol. 97, pp. 9187–9195.
33. Lisichkin, G.V., Kulakova, I.I., Gerasimov, Y.A., Karpukhin, A.V., and Yakovlev, R.Yu., *Mendeleev Commun*., 2009, vol. 19, pp. 1–2.

34. Sotowa, K.I., Amamoto, T., Sobana, A., Kusakabe, K., and Imato, T., *Diam. Relat. Mater*., 2004, vol. 13, pp. 145–150.
35. Korolkov, V.V., Kulakova, I.I., Tarasevich, B.N., and Lisichkin, G.V., *Diam. Relat. Mater*., 2007, vol. 16, pp. 2129–2132.
36. Li, L., Davidson, J.L., and Lukehart, C.M., *Carbon*, 2006, vol. 44, pp. 2308–2315.
37. Touhara, H. and Okino, F., *Carbon*, 2000, vol. 38, pp. 241–267.
38. *Rossiiskaya entsiklopediya po okhrane truda* (Russian Encyclopedia), Moscow: NTs ENAS, 2007, 2 ed., vol. 3, p. 181.
39. Karkischenko, N.N., *Biomeditsina*, 2009, no. 2, pp. 5–26.
40. Meinhardt, T., Lang, D., Dill, H., and Krueger, A., *Adv. Funct. Mater*., 2011, vol. 21, pp. 494–500.
41. Yakovlev, R.Yu., Kulakova, I.I., Leonidov, N.B., and Lisichkin, G.V., Abstracts of Papers, *5-ya Vseross. konf. s mezhdunarodnym uchastiem "Khimiya poverkhnosti i nanotekhnologiya"* (5th All-Russia Conf. with Int. Participation "Surface Chemistry and Nanotechnology"), Khilovo, September 23–30, 2012, pp. 292–293.
42. Ho, D., *Nanodiamonds: Applications in Biology and Nanoscale Medicine*, New York: Springer, 2009.
43. Huang, L.-C.L. and Chang, H.-C., *Langmuir,* 2004, vol. 20, no. 14, pp. 5879–5884.
44. Wei, L., Wei, Z., Lu, H., and Yang, P., *Talanta*, 2010, vol. 80, pp. 1298–1304.
45. Perevedentseva, E., Cai, P.-J., Chiu, Y.-C., and Cheng, C.-L., *Langmuir,* 2011, vol. 27, no. 3, pp. 1085–1091.
46. Solomatin, A.S., Yakovlev, R.Yu., Kulakova, I.I., Chernyshova, M.G., and Badun, G.A., Abstracts of Papers, 5-*ya Vseross. konf. s mezhdunarodnym uchastiem "Khimiya poverkhnosti i nanotekhnologiya"* (5th All-Russia Conf. with Int. Participation "Surface Chemistry and Nanotechnology"), Khilovo, September 23–30, 2012, pp. 272–273.
47. Yeap, W.S., Tan, Y.Y., and Loh, K.P., *Anal. Chem*., 2008, vol. 80, no. 12, pp. 4659–4665.
48. Purtov, K.V., Burakova, L.P., Puzyr, A.P, and Bondar, V.S., *Nanotechnology,* 2008, vol. 19, pp. 1–3.
49. Grichko, V., Grishko, V., and Shenderova, O., *Nanobiotechnology*, 2006, vol. 2, pp. 37–42.
50. Huang, H., Pierstorff, E., Osawa, E., and Ho, D., *Nano Lett*., 2007, vol. 7, no. 11, pp. 3305–3314.
51. Lam, R., Chen, M., Pierstoff, E., Huang, H., Osawa, E., and Ho, D., *ACS Nano*, 2008, vol. 2, no. 10, pp. 2095–2102.
52. Chow, E.K., Zhang, X.-Q., Chen, M., Lam, R., et al., *Sci. Trans. Med*., 2011, vol. 3, no. 73, pp. 73ra21.
53. Adnan, A., Lam, R., Chen, H., Lee, J., et al., *Mol. Pharmaceut*., 2011, vol. 8, no. 2, pp. 368–374.
54. Shimkunas, R.A., Robinson, E., Lam, R., Lu, S., et al., *Biomaterials,* 2009, vol. 30, pp. 5720–5728.
55. Krueger, A. and Waag, T., *Int. Conf. on Diamond and Carbon Materials*, Granada, September 3–6, 2012, p. O.1. *https://elsevier.conference-services.net/secure-Programme.asp?conferenceID=3113*.
56. Chen, M., Pierstorff, E.D., Lam, R., Li, S.Y, et al., *ACS Nano*, 2009, vol. 3, no. 7. pp. 2016–2022.
57. Solomatin, A.S. and Yakovlev, R.Yu., Abstracts of Papers, *Vseross. molodezhnaya konf. "Khimiya poverhhnosti i nanotekhnologiya" v ramkakh festivalya nauki* (All-Russia Youth Conf. "Surface Chemistry and Nanotechnology" in the framework of Science Festival), Kazan, October 9–11, 2012, p. 46.
58. Schrand, A.M., Huang, H., Carlson, C., Schlager, J.J., et al., *J. Phys. Chem*. *B*, 2007, vol. 111, no. 1, pp. 2–7.
59. Karpukhin, A.V., Avkhacheva, N.V., Yakovlev, R.Yu., Kulakova, I.I., et al., *Cell Biol. Int*., 2011, vol. 35, pp. 727–733.
60. Ferrari, M., *Nature Nanotech*., 2008, vol. 3, pp. 131–132.
61. US Patent 2009/0226495, 26.11.2008.
62. US Patent 2010/0129457, 27.05.2010.
63. US Patent 2011/0008447, 13.07.2009.
64. Ushizawa, K., Sato, Y., Mitsumori, T., Machinami, T., et al., *Chem. Phys. Let*., 2002, vol. 351, pp. 105–108.
65. Dahoumane, S.A., Nguyen, M.N., Thorel, A., et al., *Langmuir,* 2009, vol. 25, no. 17, pp. 9633–9638.
66. Krueger, A., Stegk, J., Liang, Yu., Lu, L., and Jarre, G., *Langmuir*., 2008, vol. 24, no. 8, pp. 4200–4204.
67. Wei, L., Wei, Z., Lu, H., and Yang, P., *Talanta*, 2009, vol. 80, no. 3, pp. 1298–1304.
68. Yakovlev, R.Yu., Kulakova, I.I., Leonidov, N.B., and Lisichkin, G.V., *Mendeleev Commun*., 2012, vol. 22, pp. 213–214.
69. Hartmann, M., Betz, P., Sun, Y., Gorb, S.N., et al., *Chem. Eur. J.,* 2012, vol. 18, no. 21, pp. 6485–6492.
70. Barras, A., Lyskawa, J., Szunerits, S., Woisel, P., and Boukherroub, R., *Langmuir,* 2011, vol. 27, pp. 12451–12457.
71. Liu, K.-K., Zheng, W.-W., Wang, C.-C., Chiu, Y.-C., et al., *Nanotechnology,* 2010, vol. 21, pp. 1–14.
72. Kawaguchi, H., Naito, T., Nakagawa, S., and Fujisawa, K., *J. Antibiot*., 1972, vol. 25, pp. 695–708.
73. Smith, A.L., *Applied Infrared Spectroscopy*, New York: Wiley, 1979. Translated under the title *Prikladnaya IK spektroskopiya*, Moscow: Mir, 1982, pp. 232–292.
74. Yakovlev, R.Yu., Osipova, A.S., Solomatin, A.S., Lisichkin, G.V., and Leonidov, N.B., Abstracts of Papers, *Tez. dokl. 19-go Mendelevskogo s'ezda po*

obschei i prikladnoi khimii (Proc. 19 Mendeleev Conf. on General and Applied Chemistry), Volgograd, September 25–30, 2011, p. 223.

75. Yakovlev, R.Yu., Alieva, I.B., Leonidov, N.B., Rakita, D.R., Agafonov, V.N., and Uzbekov, R.E., Abstracts of Papers, *Int. Conf. "Advanced Carbon Nanostructures",* St. Petersburg, July 4–8, 2011, p. 206.
76. Yuan, Y., Chen, Y., Liu, J.-H., Wang, H., and Liu, Y., *Diam. Relat. Mater*., 2009, vol. 18, pp. 95–100.
77. Zhang, X., Yin, J., Li, J., Zhu, Y., et al., *Toxicol. Lett*., 2010, vol. 198, no. 2, pp. 237–243.
78. Yuan, Y., Wang, X., Jia, G., Wang, T., et al., *Diam. Relat. Mater*., 2010, vol. 19, pp. 291–299.
79. Qi, W., Li, Z., Bi, J., Wang, J., et al., *Nanoscale Res. Lett*., 2012, vol. 7, no. 8, pp. 473.
80. Deryabin, D.G., Aleshina, E.S., and Tlyagulova, A.S., *Ross. Nanotekhnol*., 2011, vol. 6, nos. 7–8, 136–141.
81. Tyan, A.G., *Cand. Sci. (Med.) Dissertation*, 2005.
82. Lazarenko, L.I., *Cand. Sci. (Med.) Dissertation*, 2009.