Nanodiamonds for Biomedical Applications – Features of Interaction with Blood Components and Behavior in the Circulatory System [Review]

Elena V. Perevedentseva
P.N. Lebedev Physical Institute of Russian Academy of Sciences, 53 Leninskii pr., Moscow 119991, Russia

*** e-mail:** perevedencevaev@lebedev.ru

Abstract. The development of the application of nanoparticles for biomedical research and theranostics in many cases involves the injection of nanoparticles into the bloodstream. The interaction of nanoparticles with blood components and the circulatory system is one of the key points of the relevant study or treatment. In this review, the interaction of diamond nanoparticles with blood *in vivo* and *in vitro* is considered in terms of the nanodiamonds safety and hematocompatibility and biomedical applications. © 2022 Journal of Biomedical Photonics & Engineering.

Keywords: nanodiamond; nanoparticles; biocompatibility; hematocompatibility; blood circulation system; blood cells.

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1 Introduction

The development of nanoparticles (NP) applications for biomedical research and for theranostics often implies the NP injection into the bloodstream. Thus, the NP interaction with components of the blood and blood circulation system becomes one of the key points of the corresponding study or treatment. It determines the safety and, as a whole, the biocompatibility of NP and the effectiveness of treatment methods based on them, as well as the choice of analytical methods when using NP as a probe (biolabel, biosensor). Understanding of the mechanisms of interaction is important both for fundamental knowledge and for further development of applications [1].

All of this holds true for diamond nanoparticles – nanodiamonds (ND). ND are studied for the past 20 years in terms of their biomedical applications due to their availability in a wide variety of sizes, structural and physical-chemical properties [2–6].

Current methods of ND synthesis enable the production of ND with various combinations of properties. For biomedical applications ND particles produced by the detonation method [2, 7], high temperature-high pressure (HTHP) method [7], or laser ablation [8] are primarily applicable. The HPHT synthesis technique involves the conditions for microdiamond formation, particularly, high pressure (several GPa) and high temperature (up to ~2000 ºC) inside a chamber, to convert carbon precursor from graphite into the diamond structure. The resultant needs to be milled to obtain the particles of size from tens to hundreds nm with a good diamond structure and a relatively low non-diamond fraction on the surface [7]. Such particles can have a well detectable photoluminescence (originated from defect color centers) [2–4, 6]. With the surface chemistry controlled by a number of chemical methods [9], it makes these particles promising for bioapplications even though they are of relatively large size. As well, they have been shown to be quite biocompatible [3, 5].

Detonation nanodiamonds (DND) are produced by explosion of explosives (usually a mixture of trotyl and hexogen) with a negative oxygen balance in a large metal chamber [7]. The increase of temperature and pressure in the chamber leads to a formation of diamond phase. The resultant is agglomerates of crystallites of average size of 4–5 nm with large admixture of non-diamond carbon. Obtained DND have wide variability of the particle sizes, structure features and surface chemistry, and for biomedical applications DND must be subjected to deaggregation and purification to remove contaminants. Small size of crystallites makes DND preferable for development of bioapplications, but problem of aggregation and need for deaggregation [10], poorly controllable surface chemistry and high variability of properties at whole can complicate their use, as well as decrease biocompatibility [3, 5]. At present, methods of laser synthesis of ND by laser treatment of carbon-containing target are also of great interest. Laser

synthesis could allow getting ND of small size, but without the drawbacks of DND [11]. Varying of the parameters of the synthesis (laser radiation parameters, a refractive index of the media, a composition of special target, etc.) is used to enhance the yield of produced ND and to control their properties. The conditions to produce ND with optimal properties for bioapplications are being discussed currently [7].

The optical-spectroscopic properties of ND are tunable and can be optimized to use ND as promising photostable biomarkers with detection of 1- or 2-photon fluorescence, Raman signal, scattering [2–5], or to exploit photoacoustic emission capability [6]. The main origin of ND fluorescence is defects in the diamond lattice – color centers [2, 4]. A huge number of the color centers have been found in the diamond, but the most well studied defects for imaging applications are Nitrogen vacancies centers (NV negative or neutral centers) and Silicon vacancy center (SiV). As-prepared HTHP ND can contain a high number of the color centers; additionally, their number can be enhanced with high-energy processing [4, 12] to obtain ND with enhanced fluorescence – fluorescent ND (FND). Intense and stable fluorescence of different defects can be excited in a wide range of wavelengths and make HTHP ND a well-detectable biomarker. Non-diamond surface carbon also demonstrates some fluorescence, which depends on surface structure and chemistry. This kind of fluorescence is more characteristic for DND, although the ability of DND to host fluorescent diamond lattice defects also is studied [13, 14]. Additionally, diamond intense Raman peak from sp3-bonded carbon can be used as imaging agent for Raman mapping [3, 5]. Properties of ND crystal lattice defects can also be exploited for ND use as a contrast agent for other advanced optical bioimaging techniques [15] – Stimulated emission-depletion microscopy, correlative microscopy, etc. [6, 12], fluorescence lifetime imaging and time-gate imaging [16] as well as for magnetic resonance imaging [6, 17]. They are also considered for biosensing [18] with detection of fluorescence lifetime [19], and for so called quantum sensing based on ND fluorescent centers spin properties [20, 21], first, optically detected magnetic resonance [22].

At the same time, the structural and surface properties of ND make it possibly to modify the surface with the molecules of interests, including drugs [9, 23–25], molecules for targeting delivery [26], like folic acid for selective delivery to mitochondria [27], or antibodies to the receptor which is highly expressed in studied cancer cell [28, 29], photosensibilizers and agents for photodynamic and photothermal therapy [30–32], genetic factors [25, 33], etc. Using ND for delivery of the biologically active species allows localizing the treatment, reducing the dosage, minimizing side effects; in addition, drug resistance has been shown to be overcome when ND anticancer drug delivery [27, 34].

Thus, ND allows combining imaging (including delivery monitoring), sensing, therapeutic treatments, and is very suitable for multifunctional theranostic applications.

When using ND in theranostics, nontoxicity and biocompatibility are of fundamental importance. Biocompatibility of ND of various origins at various levels of biological systems organization has been studied. The results obtained on a large variety of cellular models looked very promising and made it possible to consider various ND as non-toxic and safe for use in sufficient high concentrations (mostly varying in range of 1–100 μg/ml) [2–6]. In cases where the investigation or medical treatment implies the administration of medicine through injections, ND in complex with attached molecules can interact with different parts of the blood system and all relevant effects must be taken into account.

2 *In vivo* Studies of ND Biodistribution, Interaction with Components of Blood Circulation System and Effects on Blood Parameters

The studies designed for animal models included analysis of biodistribution, further long-term fate in the organs and selected adverse effects on various body systems. Studies of ND effects on blood characteristics are summarized in the Table 1.

For different animal models ND after intravenous injection were found predominantly accumulated in liver, spleen and lungs [35–39], and in smaller amounts they could be observed in other organs (Fig. 1). About 60% of ND of average size 50 nm injected intravenously into mice were found in the liver from 0.5 h to 28 days post injection [24]. No induced inflammatory processes were detected in mice models within 3 months after subcutaneous injection of ND. Intravenous administration of high concentration of detonation ND did not cause the lethality in a rabbit model [40], but could affect a number of blood biochemical parameters [38–43]. Also in the mice model [41], ND after injection was detected in the liver up to 97 days, while only in the first 2.5 h after the injection, the Activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the level of bilirubin in the blood (which are used to identify toxins in the liver, liver disease or damage) increased and then decreased to the initial values.

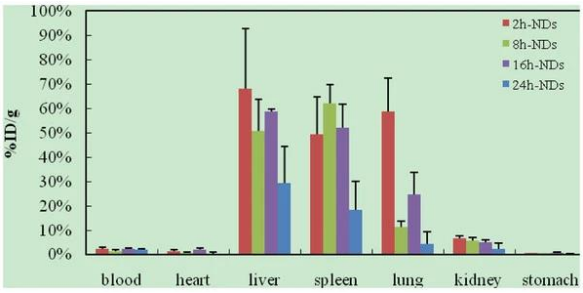


Fig. 1 Biodistribution of ND in mice at 2, 8, 16, and 24 h post intravenous ND were radiolabeled with technetium-99 (^{99m}Tc) for the organs loading counts. Reproduced with permission from Ref. [39].

Table 1 Studies of influence of ND on blood parameters at *in vivo* administration.

Blood parameters	**Animal model/ND concentration/periodicity**	**References**
RBC: count	Mice (DND 0.2–0.5 ml of 0.1–5 wt%*), rabbit (20 ml of 0.1–5 wt%);	[40]
	Rats (DND 1–2 mg per 139–155 gBW**, twice during 2 weeks), Cynomolgus monkeys (15–25 mg/kgBW; 1 time per month for 6 months)	[38]
Hb level / hematocrite	Rats (DND 1–2 mg/139–155 gBW, twice during 2 weeks), Cynomolgus monkeys (DND 15–25 mg/kgBW; 1 time per month for 6 months)	[38]
RBC superoxide dismutase activity, glutathione reductase, and glutahione peroxidase activity	Rats (DND 1 mg/kgBW, once)	[42]
WBC count	Rats (DND 1–2 mg/139–155 gBW, twice during 2 weeks), Cynomolgus monkeys (DND 15–25 mg/kgBW; 1 time per month for 6 months)	[38]
Platelets count	Rats (DND 1–2 mg/139–155 gBW, twice during 2 weeks), Cynomolgus monkeys (DND 15–25 mg/kgBW; 1 time per month for 6 months)	[38]
	Rats (DND 1 mg/kgBW, once)	[44]
Neutrophils	Mice (DND, 1 wt%)	[88]
	Cynomolgus monkeys (DND 15–25 mg/kgBW; 1 time per month for 6 months)	[38]
Cytokine level	Rats (DND 1 mg/kgBW, once)	[42]
Tumor necrosis factor α,	Mice (HTHP oxidized ND, PA***-ND HP****-PA-ND; 2 mg/kg)	[51]
Clotting time, Fibrinogen level	Rats (HTHP carboxilated ND, 20 mg/kg BW)	[78]
	Rats (DND 1–2 mg/139–155 gBW, twice during 2 weeks), Cynomolgus monkeys (DND 15–25 mg/kgBW; 1 time per month for 6 months)	[38]
Blood plasma chemistry: AST, ALT activity Bilirubin, lipid methabolism, glucose, etc.	Rats (DND 1 mg/kgBW, once), Mice (DND 0.2–0.5 ml of 0.1–5 wt%, once),	[40, 41, 44]
Total antioxidative state	Rats (DND, 1mg/kgBW, once)	[42]

* wt% – weight percents; ** BW – body weight; ***PA – polyarginine; ****HP – heparin

The immune response to different types of ND was also tested. The effect of detonation ND on redox and immune parameters in rats upon intravenous and intraperitoneal injection was observed. At the same time, small-sized detonation ND did not directly affect the antioxidant characteristics, but significantly interfered with the organism defense against the formation of reactive oxygen species and the inflammatory response through inhibiting the activity of innate immune cells [42].

Larger ND produced by HTHP method (50 and 100 nm) injected into mice via the caudal vein did not stimulate the immune response [43]. Significant decrease in glucose and total protein levels and elevated platelet count parameters were observed after intravenous injection of a relatively high dose of ND [44]. In contrast

to Ref. [43] the immune response consisting in a significant increase in tumor necrosis factor (TNF)-α and TNF-α and interferon IFN-γ protein levels measured in peripheral blood mononuclear cells was observed after stimulation with bare ND [45]. Note, however, that no the response was observed when the same cells were exposed to RNA-coated ND.

In intratracheal administration, ND can reveal not only a respiratory toxicity, but also an air-blood barrier penetration. After that DND (with crystallites of size 2–10 nm) was found distributed not only in lungs, but also in the spleen, liver, bone and heart and could induce dose-dependent effect on the organs observed via histological analysis of morphology [46]. After oral administration detonation ND also can affect hematological parameters (blood cells count) as well as the state of liver cells of rats *in vivo* [47]. In contrast to detonation ND, the particles of small sizes, but obtained by HTHP method with subsequent crash to the sizes 4 nm and 50 nm, did not induce measurable pulmonary toxicity; and no oxidative stress was observed [48].

ND surface functionalization [49–51] and coating [52, 53] with macromolecules by adsorption or conjugation can affect the ND *in vivo* safety [54, 55], as well as distribution and predominant localization in organism. Thus, in the sentinel lymph nodes of both healthy mice and mice with B16 melanoma, an increased accumulation of fluorescent ND stabilized by alkyne-functionalized polyglycerol and modified by the surface with a polyvalent set of mannoses was observed [56].

Various methods allow the detection of ND to clarify the entry into different body systems. The radiolabeled with ^{18}F [37] or ^{99m}Tc [39] ND were administered to mice and rats and detected using radioactive labels accumulated mainly in the lungs, and additionally in reticuloendothelial system of liver and spleen, as well as were found excreted into the urinary tract [37]. Alternative method of detection was applied for 100 nm fluorescent HTHP FND to test ND long-term stability in the body. In this case the ND biocompatibility for rats was observed within 5 months after injection [56]. Both *in vivo* and *ex vivo* fluorescence imaging has made it possible to visualize FND distribution, particularly, in lymph nodes. At the same time no harmful effects were observed at high dosage and long-term exposure to FND of different sizes [57, 58]. This is consistent with *in vivo* studies of ND in non-human primates; the animals were doing well for 6 months [38]. Body and organ weight, motor and sensory function tests, blood cell counts, kidney and liver function and functions were controlled and remained normal.

Thus, *in vivo* biocompatibility studies show that injected ND circulated in blood system within tens of minutes post injection [58] and then accumulated in mainly liver, lungs, spleen, but mentioned above sensitive methods of detection find additional localizations [37, 39, 57, 58]. Also, ND can penetrate in blood and circulate in the blood system even after respiratory administration. A number of studies have shown safety of ND at *in vivo* administration. However, dose-dependent effects on some body parameters can be observed. It is important that ND can be extracted in the urine, since the long stay of ND after administration in the organism is one of the main limitations of theranostic use of ND.

Some discrepancy between the results of different studies is probably due to a significant difference in the properties of the used ND (depending, in particular, on the size [59] or on the functionalization and conjugation of the surface [60, 61]) and with different effects on the selected parameters for the organism state analysis. All this should be taken into account when developing any ND bioapplication. In addition, ND distribution in organism can be affected by the organism state. Thus, it has been shown that inhaled NP accumulate at sites of vascular disease in vessels (e.g. in area of atherosclerotic plaque) [62]; a predominant accumulation of polyglycerol-functionalized ND [61] and hybrid ND-lipid particles [53] in the tumor site was observed and attributed to stealth effect of polyglycerol which allows avoiding recognition by reticuloendothelial cells and excretion [61].

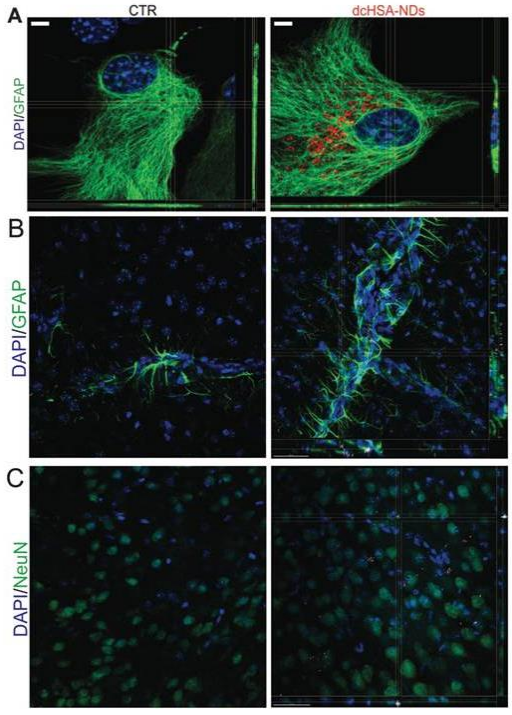
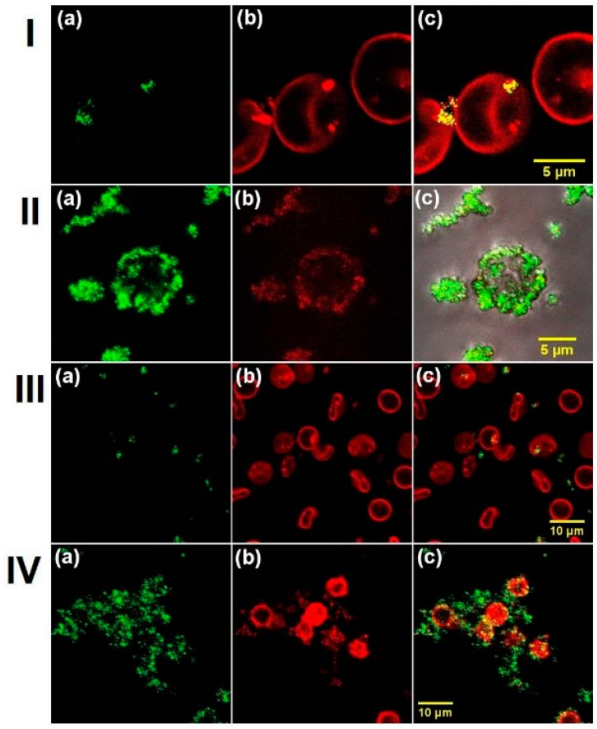


Fig. 2 (A) NDs stabilized by a protein-derived biopolymer coating (polyethylene glycolylated denatured human serum albumin; dcHSA-PEG) are taken up by neurons and astrocytes *in vitro*. Representative confocal orthogonal views of dcHSA-NDs (red) uptake in astrocytes labeled by the serum Glial Fibrillary Acidic Protein (GFAP) marker (green). *In vivo* fluorescent dcHSA-NDs cross the BBB and are tracked on single cell level in neurons and astrocytes: Representative confocal orthogonal views of dcHSA-NDs (white) signals in brain slices: localization in GFAP-labeled astrocytes (green) (B) and NeuN-labeled neurons (green) (C); scale bar = 40 μm [67]; left panel – control, right – dcHSA-ND treated samples. Reproduced with permission from Ref. [67].

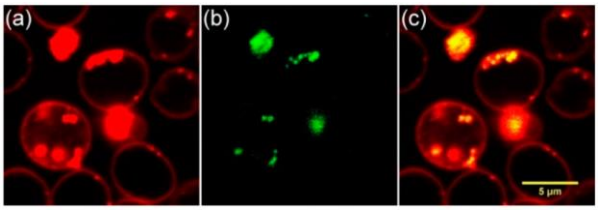
Speaking about the distribution of NA in the organs of a living organism, it seems necessary to touch upon the important issue of NA penetration through the blood-brain barrier (BBB) [63]. The BBB separates brain circulation from blood-borne agents, regulates serum transport, protects the central nervous system from infections, neurotoxins, and exogenous compounds, but also prevents drugs or markers penetration [64]. The discovered ability of ND to penetrate the BBB opens up new possibilities for delivery of medicines for the treatment of brain deceases [23], including brain cancer, neurodegenerative and infection diseases. Efficient transport of biopolymer-coated ND across an intact BBB was observed using *in vitro* model [65] and after intravenous injection *in vivo* [66]. Fig. 2(a) shows images of neurons and astrocytes engulfed *in vitro* with ND stabilized by a labeled with fluorescein marker Bodipi488 protein-derived biopolymer coating (polyethylene glycolylated denatured human serum albumin with; dcHSA-PEG). Administrated *in vivo* the fluorescent dcHSA-ND crosses the BBB and is tracked on single cell level in neurons and astrocytes: dcHSA-NDs uptake is observed in astrocytes labeled by the serum Glial Fibrillary Acidic Protein (GFAP) marker (green) and 4,6-diamidino-2-phenylindole (DAPI) marker (blue) (Fig. 2(b)) and neurons labeled with neuronal nuclear protein (NeuN) marker (green) (Fig. 2(c)).

Both, crossing BBB and targeting affected areas of the brain are discussed in terms of the use of ND to understand the etiology of neurodegenerative diseases and search for effective methods for delivering neurodrugs to certain areas of the brain [67, 68]. For example, 4-Aminopyridine, a nonselective blocker of potassium channels, used in clinical practice for the symptomatic treatment of demyelinating diseases, was delivered with ND into the neocortex of rat brain [69]. This work demonstrated a technique for a local administration and efficient action of very small amount of the medicine, with the slow and gradual release. Crossing BBB was a necessary condition for observing the neuroprotective effect of intraperitoneally injected ND in aluminum-induced Alzheimer disease-like cognitive deficits in rats [70]. ND injection improved learning and memory tested in mice, reversed histological alterations and reduced pathological aberrations at the molecular level. The central nervous system is also reservoir organ for Human Immunodeficiency Virus Type 1 (HIV-1). Using an *in vitro* model of the BBB, an increase in the effectiveness of the treatment of neurological disorders associated with HIV-1 using ND for the transport of anti-HIV-1 drugs through the BBB was demonstrated [71].

As a result of interaction of NP with red blood cells (RBC, erythrocyte) *in vivo* the adsorption of various (in particular, polymeric) NP on the RBC membrane and circulation together with the RBC can be observed [72]. RBC circulating in the blood system for 90–120 days can be considered as a natural vehicle for drug delivery. Attachment of NP on the RBC membrane increases the circulation time of NP, reduces rapid clearance [73], which improves a targeting and efficiency of slow drug release. This facilitates the drug delivery in the brain and the crossing of the BBB [66, 74]. Mostly polymeric NP are usually used [75]. However, it also was demonstrated that ND can attach to RBC membrane (Fig. 3) – this has been shown *in vivo* and *in vitro* [43] – and circulate together with RBC.



(A)



(B)

Fig. 3 (A) Fluorescence image of RBC with ND. Human RBC with 100 nm ND (I: 20 μg/mL; II: 1000 μg/mL); rat RBC with 50 ND (III: 20 μg/mL; IV: 1000 μg/mL); (a) ND were excited by a 543 nm wavelength laser, and the signal was collected in the 610–640 nm region and shown in green; (b) the RBC membranes were dyed with 3,3-dipentyloxacarbocyanine iodide (DIOC5), excited by a 488 nm wavelength laser, and the signal was collected in the 500–530 nm range, shown in red; (c) merged images of (a) and (b). (B) Confocal images: *in vivo* circulation of ND in rat. (a) The stained RBC membrane is shown in red; (b) ND fluorescence is shown in green; and (c) the merging of (a) and (b) [43]. Reproduced with permission from Ref. [43].

In general, analyzing ND distribution in organism, it can be concluded that the circulation of ND with blood can make it possible to deliver the drug to target sites, including the brain drug delivery to target sites, including the sites in the brain. ND administrated in concentrations 1 – 15 mg/BW (for different animal models) and accumulated in the body does not reveal a pronounced toxic effect and can be gradually/particularly exerted from the organism with urine.

3 *In vitro* Studies of the Interaction of ND with Blood Cells and Other Blood Components

While ND is generally considered biocompatible, some parameters of blood/organism may be altered by administering ND to the body through the blood, as noted above. The influence of ND and potential detrimental effects on blood system components such as blood cells or blood plasma is the subject of detailed *in vitro* studies. Note that *in vitro* studies on the nanoparticles-blood interaction are important also because, unlike *in vivo* animal experiments, human blood experiments can be performed (Table 2).

Really the earliest studies of the interaction of ND with biological samples and assessment of their biosafety were carried out on blood cells. In early works, the destruction of white blood cell (WBC, leukocytes) and hemolysis of red RBC, as well as change the kinetics of active oxygen generation were observed when a suspension of blood cells was treated *in vitro* with DND [76, 77]. Thus, early publications demonstrated the harmful effects of ND on blood cells *in vitro* and on experimental animals *in vivo*. The authors suggested that the treatment conditions for ND were not optimal. Indeed, more recent numerical studies of ND biocompatibility have demonstrated the use of ND both *in vitro* and *in vivo*, have discussed and found conditions for safe applications [78–87].

Some *in vitro* investigations of the effect of ND effects on whole blood [49, 50], certain characteristics of blood cells and their functions or other blood system components did not find any deviations [78, 79], while some other properties can reveal relatively weak alterations [48, 51, 76–83]. Thus, strong aggregation of detonation ND is one of the limiting factors of its bioapplications, but it has been shown that even partially disaggregated carboxylated DNA does not affect either the viability of RBC or the Raman spectra of hemoglobin (Hb) [79]. In more detailed investigations on the influence of different ND on RBC oxygenation and deoxygenation dynamics [43, 80] it was shown that HTHP ND of size of 100 nm and detonation ND did not affect human RBC viability *in vitro*, as well as no structural transformations in oxygenated or deoxygenated Hb were observed using Raman spectroscopy. But detonation ND still had slight effect on the oxygenation and deoxygenation dynamics. The study of ND interaction with rat blood *in vitro* showed that adsorption of ND on the RBC membrane did not affect the oxygenation state of RBC when the ND concentration in the medium did not exceed 100 μg/ml [43]. However, the ND adsorption on RBC membrane can alter mechanical and, accordingly, the rheological properties of RBC. It was shown that ND could reduce the deformability of RBC and increase aggregation in a concentration-dependent manner [80]. It is consistent with the results of studies of the mutual interaction of RBC under the influence of ND [81], when an increase in adhesion forces is observed during the interaction between RBC in the presence of ND. This led to the formation of larger RBC aggregates and influenced the shape of the RBC. In addition, there was no significant alteration in the hemolysis of RBC exposed to ND [45, 80], as well as in RBC from whole blood incubated with oxidized or hydrogenated ND [49] – the ND affected no more than 3% of cells.

From the analysis of the processes of oxygenation and deoxygenation of RBC, it can be seen that the effect of detonation ND is more significant than for HTHP ND of larger size. Consistent difference could be observed when studying the effect of various ND on the blood coagulation system. Carboxylated detonation ND with a crystallites size of 4–10 nm [82] induced significant platelet activation, stimulated the formation of a prothrombotic platelet phenotype, increased binding of annexin V on the platelet surface, the formation of reactive oxygen species, and disruption of the mitochondrial transmembrane potential. This ND, administered intravenously to mice, was found to cause massive pulmonary thromboembolism. Lung tissue images revealed a large number of vessels occluded by platelet thrombi with ND [82]. However, for ND with a size of 100–140 nm at various concentrations, no effect was observed on blood coagulation parameters analyzed using the activated partial thromboplastin time (aPTT) test [78], and coagulation in the presence of ND was initiated through the intrinsic pathway. High biocompatibility has been demonstrated for carboxylated ND (ND-COOH) and amino-functionalized ND (ND-NH_2) using human peripheral blood mononuclear cells (PBMC). Both NDs slightly affected the immune response, however carboxylated ND revealed more prominent responses, affecting a regulation of immunomodulatory transcripts [83].

In whole blood, ND interact not only with blood cells, but also with blood plasma; in particular, macromolecules from plasma adsorb on ND surface [84, 85]. A number of blood plasma proteins were adsorbed on the surface of ND [51–55, 86, 87]. However, the adsorption of blood plasma proteins on bare ND can affect the concentration of plasma components and osmotic properties [76], changing the properties of the blood as a whole. On the other hand, the structure of blood serum proteins, for example adsorbed albumin, can also be affected by surface of ultrafine ND [86, 87], which can lead to a change in the functional properties of proteins. Thus, preliminary coating with ND reduces the undesirable effects of the ND on the blood, increases biocompatibility, allows avoiding uncontrolled adsorption, and, overall, makes it possible to optimize the interaction of the ND-based complex with the target or with blood [54].

Table 2 *In vitro* studies of influence of ND on blood and blood components.

Blood component	**ND, functionalization**	**Analyzed effect/interaction**	**Method**	**Ref.**
Whole blood: human	Ultrafine DND	Generation of active oxygen species produced by WBC	Chemiluminescence	[76, 77]
human	DND	RBC hemolysis	Spectrophotometry	[76, 77]
Human blood	MW/PASVD*-modified DND	RBC morphology and aggregation	Microscopy	[49, 50]
WBC (human)	DND	Viability	Trypan blue test	[77]
Mice neutrophils	DND	Viability, ROS*** generation, cell viability, counting, activation	Trypan blue test, Chemiluminescence, Flow cytometry, Immunostaining, Immune Gene Array, Cytokine secretion assay	[88]
Human monocytes	DND; COOH-DND; NH_2-DND; FITC**-DND			[83]
RBC: Human	HTHP: oxidized ND, PA-ND; HP-PA-ND	Hemolysis	Spectrophotometric assay	[51]
Human	DND, COOH-DND, NH_2-DND, FITC-DND	Hemolysis	Hb absorption	[83]
Human	Carboxylated DND, Carboxilated HTHP ND	Aggregation	Light scattering	[80]
Human	Carboxilated HTHP ND	Aggregation	Optical tweezers	[81]
Human	Carboxilated DND&HTHP ND	Deformability	Light scattering	[80]
Human and rat blood	Carboxylated DND & HTHP ND HAS-ND	Oxygenation-deoxygenation dynamics	Raman scattering	[43, 80]
Human platelets	Carboxylated DND	Platelet aggregation, Platelet activation	Electron Microscopy, Immunostaining / fluorescence analysis	[82]
Human plasma clotting factors	HTHP: oxidized ND, PA-ND; HP-PA-ND	Cell viability, counting, activation (immunogenes, cytocines)	aPTT* test	[51]
Human	Carboxylated HTHP ND, ND-albumin	Thrombogenicity	aPTT test	[78]
Human plasma biochemistry	EDTA**-modifies DND	Glucose, urea, bilirubin, creatinine, uric acid, total protein, albumins, and etc., concentrations	Biochemical analyzer / reagent kits for biochemical investigations (Vital Diagnostics SPB)	[85]
Human blood vessels, endothelial cells (HUVECs**)	HTHP: oxidized ND, PA-ND; HP-PA-ND	Cell viability	CCK-8*** assay	[51]

MW/PASVD* – microwave/plasma activated chemical vapor deposition method; FITC** – Fluorescein isothiocyanate; ROS*** – reactive oxygen sites; aPTT* – activated partial thromboplastin time; EDTA** – Ethylenediaminetetraacetic acid; CCK-8*** – Cell Counting Kit.

4 New Applications of "Bare" ND

Obviously, most of bioapplications imply the use of a complex of ND with molecules of interests. However, the effects of non-modified / uncoated bare ND on biological systems are also being studied to understand the interaction mechanisms and ways of the ND use [2–6]. Moreover, some applications utilizing the effects of non-modified / uncoated bare ND on the biological systems are also considered. This section discusses some applications of the bare ND for the study and treatment of blood and blood components. Thus, the use of ND for controlled modification of the inflammatory and immune response is discussed [88–90]. It has been observed that detonation ND modifies the activity of neutrophils from the site of acute inflammation. When ND is used at concentrations less than 1 g/l, the activity induced by bactericidal agents increases, and an increase in concentration prevents excessive cell activation during phagocytosis [88]. The ability of ND to enhance the immune response against the recombinant protein of Influenza A virus (HA / H7N9) was observed. The response was demonstrated by an increase in the amount of H7N9-specific immunoglobulin IgG. The possibility of using this effect to develop an effective nanovaccine against the H7N9 Influenza virus is discussed [89]. On the other hand, uncoated highly purified detonation ND was applied as a cytokine "sponge" in infectious diseases [90]: intravenously administered ND has been proposed a therapeutic agent for treatment of cytokine release syndrome (CRS – a systemic inflammatory response resulting in overexpression of cytokines, which leads to poliorganic failure) by adsorbing inflammatory cytokines. Successful ND inactivation of key cytokines in the blood plasma of CRS patients with COVID-19, pneumonia and septic shock has been demonstrated [90]. Intravenous injection of ND in a mouse sepsis model improved survival rates and prevented tissue damage by reducing blood-circulating inflammatory cytokines. As far as non-infection deceases are concerned, ND has been shown to help regulate protein imbalances and protein–protein interactions in the blood of patients with acute ischemic stroke [91]. Using the fluorescent ND as optical labels for wide-field time-gated fluorescence imaging and flow cytometry to detect cancer cells in the blood was proposed [92].

The sensing abilities of ND are based on fluorescence and spin properties of color centers (for example, NV centers) and, in general, can be used to develop nano-tools for highly local measurements of temperature, pH, etc., in biological samples, including blood [20]. From the point of view of blood testing, the use of color centers in ND for the sensitive detection of metalloprotein molecules (in particular, heme-containing ones) may be promising [93].

The protective effect of ultradispersed ND against ionizing irradiation, which can create oxidative damage and destruction of RBC membrane (and, in addition, a decrease of oxygenation of hemoglobin), was observed for RBC *in vitro* for stored gamma-irradiated blood [94] and *in vivo* for cancer rats irradiated with X-ray [95].

In addition to the aforementioned applications for drug delivery and immune response enhancement, the direct antimicrobial activity recently observed in ND is also considered. Thus, the use of ND as therapeutic agent against various pathogens, including bloodstream pathogens, is now discussed [96]. Inter alia, bactericidal effect of ND was observed for *Staphylococcus Aureus* [97], sorption of hepatitis B and C viral particles from the blood plasma of patients [98], as well as of A(H1N1), A(H1N1)v, A(H3N2) and B influenza viruses [99] have been demonstrated.

5 Conclusion

A large variety of ND in size, structure and physico-chemical properties is determined by various methods of their synthesis, as well as by a number of developed methods of modifying and optimizing properties. Therefore, ND is highly attractive for development of applications for biomedical research and for theranostics. Such applications require an understanding of how ND interacts with biological systems. The mechanisms of interaction are investigated; properties of ND that can be used for development of bioapplications and the characteristics that determine the ND biocompatibility are analyzed in variety of biological models, including cellular cultures, tissues, and organs, up to the whole organism.

ND bioapplications often involve their injection into the bloodstream, so many studies focus on the hematocompatibility of ND, including mechanisms of interaction of ND with the blood, post-administration biodistribution in the body, and others. It has been shown that injected ND circulate in the blood for tens of minutes, and then accumulates in the organs primary in the reticuloendothelial tissue, accumulation in the lymph nodes and penetration through the blood-brain barrier are also observed. Concentrations of ND can be chosen and ND surface coating can be designed to provide safe and prolonged circulation *in vivo*. These results regarding the behavior of ND in the blood system and the interaction with the blood components, as well as the physical and chemical properties of ND, provide opportunities for the development of ND multifunctional applications that can combine imaging, sensing with drug delivery and some prospective therapeutic options.

Disclosures

The authors declare no conflict of interest.

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