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Myramistin adsorption on detonation nanodiamonds in the development of drug delivery platforms

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ABSTRACT

Detonation nanodiamonds (NDs) are considered to be potential carriers in drug delivery platforms because of their low cytotoxicity (4–5 nm particles), biocompatibility, highly developed surface area, chemically inert core and intrinsically hydrophilic surface. The adsorption/desorption processes of physiologically active compounds on ND surfaces play an important role in drug design. The electrostatic attraction between ND surface groups and the functional groups of adsorbates is the most commonly described mechanism of the adsorption process. However, the role of hydrophobic interactions has not yet been described. Here, we show that a positively charged antiseptic agent (Myramistin®) binds to a ND possessing a positive zeta potential via hydrophobic interactions between the hydrocarbon fragment of Myramistin and graphitic carbon of the ND surface. To achieve a reliable determination of low values of adsorption, a radioactive label was introduced into Myramistin molecules. Tritium was used as a labeling agent and it was introduced into Myramistin by means of tritium thermal activation method. The obtained value of the binding strength indicates easy drug release, as confirmed by in vitro release studies. We anticipate that our assay and the results obtained to be a starting point for more sophisticated models of drug adsorption/desorption processes of nanodiamond drug delivery platforms.

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1. Introduction

Detonation nanodiamonds (NDs) are considered to be prospective carriers in drug delivery. The surface of NDs contains primarily –OH, $-CH_3$, $-CH_2$, CO_2 and –C═O groups [1]. Furthermore, NDs are optically transparent and capable of fluorescing from point defects [2]. The surface can be functionalized with various agents, including radioactive labels [3–5] and compounds with biological activity [6]. In the literature [7,8], NDs are described as a material with great potential for biomedical applications because of their unique physical and chemical properties, such as a small particle size (monocrystalline samples of 4 to 5 nm), large specific surface, chemically inert core and intrinsically hydrophilic surface. The small particle size of NDs provides potential applications in drug delivery, protein immobilization and biosensors.

Modification of the ND surface with biologically active compounds and drugs offers a potential application for NDs in biosystems. The most commonly tested systems combine NDs and therapeutic agents, such as doxorubicin (DOX), daunorubicin or cisplatin. Anticancer agents can functionalize the surface of nanomaterials via chemical activation of surface groups or by non-covalent binding [9,10]. Adsorption is one of the basic mechanisms by which anticancer agents bind to NDs [11–15]. Physical chemistry studies concerning microscopic properties have confirmed that electrostatic interactions have a key role in the formation of agglomerates of primary particles that are also critically important for binding polymers and drugs [16,17]. Huang et al. obtained complexes of ND-DOX by adsorption in the presence of NaCl [14]. They proposed a salt-mediated mechanism that balances positively charged DOX ions with Cl^- ions to reduce the repulsion between DOX–DOX and ND–ND that occurs in the absence of salt. ND released DOX under acidic conditions when H^+ weakens the electrostatic attraction between the surface carboxyl of ND and the amino groups of DOX [15]. The pH dependence of DOX adsorption on NDs was also confirmed by computer simulation that suggested that the binding of DOX molecules on NDs requires at least 10% of the ND surface area to be fully available for binding and occurs only at high pH [18]. Xi et al. [19] studied DOX–ND complex uptake by glioma cells. They showed that doxorubicin uptake and retention were prolonged and significantly enhanced through conjugation with NDs on the premise that the ND-mediated delivery limited doxorubicin distribution in normal tissue and reduced toxicity. Enhancement of chemotherapeutic agents after adsorption on NDs was also demonstrated for daunorubicin [20]. In the cited paper, it was shown that NDs improve drug delivery into a resistant leukemia cell line. Thus, NDs are considered to be carriers with a demonstrated potential to improve the efficiency of cancer treatment, especially towards resistant strains.

Development of drug delivery platforms utilizing nanodiamonds requires colloidal stability in a physiological medium. The stability of ND suspensions can also be increased by the addition of surfactants [21–23]. Thus, medical applications that use drugs, which can also

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function as surfactants, for the non-covalent modification of ND surfaces look promising. Benzyldimethyl–myristoylamine–propylammonium chloride monohydrate is commercially available in Russia as Myramistin®, which is a broad-spectrum antiseptic agent. The chemical structure of Myramistin is presented in Fig. 1.

The compound belongs to a group of organic ammonium compounds that possess antimicrobial, antifungal and antiviral effects themselves [24] and as part of other medicines [25–27]. The antiviral activity of both Myramistin and modified NDs [28,29] suggests a clear potential for the antimicrobial activity of the conjugate. In addition to possessing a broad-spectrum antiseptic activity, Myramistin possesses a high surface activity that can enhance binding capacity [30]. Our previous experience shows that the aggregation stability of modified NDs can be enhanced when the ND surface coverage by modification agents does not exceed a monolayer [31]. The goal of this research was not to design a new conjugate but to focus on the adsorption/desorption stages in the development of the drug delivery system. To this end, we conducted adsorption experiments with detonation nanodiamonds and Myramistin and analyzed the aggregation stability of conjugated particles and their stability in a serum albumin solution.

2. Experimental section

2.1. Materials and characterization

Myramistin® was purchased from Infamed (Moscow, Russia) and used as received. For the adsorption study, it was labeled with tritium by a tritium thermal activation method [32]. Labeling and purification procedures are described below.

Detonation ND powder was a product of Sinta (Belarus), characterized with IR-spectroscopy and used without further purification. The infrared spectrum was recorded from 520 to 4000 cm^{-1} using a Protege 460 (Nicolet) instrument. The spectrum was recorded in a KBr pellet, prepared by pressing a mixture of 50 mg of ND powder with 1.5 mg KBr.

The specific surface of this ND was previously characterized and is approximately 310 m^2/g [33].

2.2. Preparation of the suspension and its characterization

Forty milligrams of ND powder was suspended in 10 mL of water that was purified by a Milli-Q system. The suspension was sonicated for 3 h using a sonic bath with a rated power of 110 W and was then incubated at 4 °C for 24 h; some of the sample precipitated. The upper phase of the mixture was separated and concentrated to dryness using a rotary evaporator. The dry sample was weighed and resuspended in Milli-Q water using sonication. The concentration of the final suspension was approximately 5 mg/mL that was determined by UV. Such prepared suspensions were stable for several weeks. For UV-analysis the suspension was diluted 100 times to achieve a concentration appropriate to that the Bouger–Lambert–Beer law performed. The technique of the determination of ND concentration in the aqueous solutions by UV is based on the fact that the absorption spectra of nanodiamond suspensions obey the Bouger–Lambert–Beer law, which ensures the precise determination of the total mass concentration of unknown nanodiamond suspension using a calibration plot [34]. In this work we used the calibration plots obtained at wavelength of 220, 250, 280, 310 and 350 nm ($l = 1$ cm). Coincidence of the results obtained at different wavelength illustrates the appropriateness of the technique. The suspensions for calibration and of unknown concentration have to be prepared similarly with the control of the particle size.

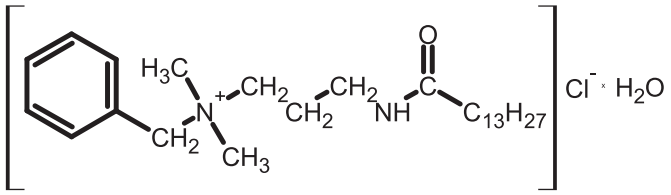

Fig. 1. Chemical structure of Myramistin.

The suspension was characterized using dynamic light scattering (DLS) by a Malvern Zetasizer Nano (Malvern Instruments Ltd., UK) instrument for the detection of size, volume and number distribution as well as zeta potential of the particles. Measurements were conducted with a detection angle of 173°. All measurements in this study were taken at a temperature of 25 °C. At least three measurements of each sample were taken to check for result repeatability. The intensity size distributions were obtained from analysis of the correlation functions using the Multiple Narrow Modes algorithm in the instrument software.

TEM images were obtained using a JEM-2100F transmission electron microscope.

2.3. Myramistin tritium labeling

Tritium labeling was carried out according to the tritium thermal activation method [32]. In this method tritium atoms can substitute hydrogen in any possible position of the molecule. Thus, the procedure of labeling includes tritium bombardment, purification from the labile tritium (the label in the functional groups) and purification from radioactive by-products. Briefly, a Myramistin® solution that contained 0.5 mg of the compound was uniformly distributed on the walls of the reaction flask and lyophilized. Then, the reaction flask was connected to the device designed for gaseous tritium and filled with tritium–hydrogen mixture up to 0.5 Pa after evacuation. Tritium content in the mixture was 27%. Tritium thermal activation occurred on the surface of W-wire disposed in the center of the flask and heated to 1800 K by the electric current during 10 s. To prevent the thermal destruction of Myramistin molecules, the flask was cooled with liquid nitrogen during the entire experiment.

After the reaction, residual gas was pumped out, and Myramistin was dissolved in 4 mL of Milli-Q water. The initial radioactivity of the sample was measured using a liquid scintillation spectrometer, RackBeta 1215 (LKB, Finland). To separate [^{3}H]Myramistin from the labile tritium (tritium in the functional groups), the solution was dried using a rotary evaporator and redissolved in water. The radioactivity loss during this procedure was approximately 47%. Thin layer chromatography (TLC) conducted on Aldrich silica plates with mixture of chloroform:ethanol:ammonia (4:7:2, v/v/v) as a mobile phase was used for product analysis and purification. The position of Myramistin on the TLC plate was monitored by UV and the Dragendorff reagent. TLC was run with a radioactive control to reveal radioactive by-products of the reaction.

To separate [^{3}H]Myramistin from radioactive by-products, preparative TLC was conducted according to the identical procedure as with the analysis. The silica of the TLC plate that contained [^{3}H]Myramistin was scraped and washed with ethanol, and the radioactivity of the ethanolic solution was measured. For adsorption experiments, a sample of the ethanolic solution was completely evaporated and the solid compound was dissolved in water.

2.4. Myramistin adsorption on nanodiamonds

For the adsorption experiments, a solution of tritium-labeled Myramistin with a concentration of 6.6 mg/mL and specific radioactivity of 0.17 MBq/mL was used. To prepare the series of Myramistin solutions of different concentrations and specific radioactivities this solution was mixed with an aqueous Myramistin stock solution and a ND suspension in 2 mL polyethylene tubes. The volume of the final suspension did not exceed 1 mL. Experiments were conducted for Myramistin concentrations ranging from 0.1 to 6 mg/mL (0.23 to 13.7 mmol/L). Suspensions were incubated at 25 °C for 24 h, followed by centrifugation. The upper phase was removed and its radioactivity was measured. The

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