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Control of nanodiamond-doxorubicin drug loading and elution through optimized compositions and release environments

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ABSTRACT

Nanodiamonds (NDs) have been extensively explored in biomedical applications due to their favorable properties, such as biocompatibilities and diverse surface electrostatics that allow rapid conjugations with drugs. Enhancing chemotherapeutic efficacy through optimized nanodiamond drug delivery system would further improve the treatment of cancers. In this study, carboxylated nanodiamonds and original nanodiamonds were involved to explore the maximum efficiency in binding and release capability of doxorubicin (DOX). The binding ratio of NDs and DOX, pH values, and release time were optimized. Notably, an NDX synthesized in 2 mL ND, 5 mL DOX, and 0.061 mL NaOH exhibits a ~63.4% (31.7 wt%) binding efficiency, and NDX release profile quantified in acidic environment exhibits ~99.9% release efficiency of DOX. The present work establishes a foundation for future applications in drug delivery system for enhancing chemotherapy efficacy.

1. Introduction

Chemoresistance and drug efflux are the key barriers to cancer treatment. Annually, chemoresistance and drug efflux account for ~35% of treatment failure in all diagnosed patients [1]. The mechanism of chemoresistance caused primarily by energy dependent multidrug resistance (MDR) and adenosine triphosphate–binding cassette transport proteins is a major limiting factor of successful chemotherapy treatments [2]. Drug efflux caused by transport proteins drastically decreases the therapeutics efficacy and correlates to the resistance in chemotherapy [3]. Hence, a critical challenge is the ability to deliver sufficient amount of drug to the diseased location avoiding consumption by those proteins [4]. With the involvement of nanotechnology, the application of nanoparticles as carriers for drugs that reduce efflux has attracted wide attention [5]. Among many other drug delivery systems developed recently, nanodiamond-mediated drug delivery system appears to be the most clinically-relevant delivery system [6–10].

Nanodiamonds (NDs), carbon octahedral structures that range from 3 to 10 nm in diameter, exhibit unique surface chemistries that allow diverse electrostatic interactions for high affinity binding to compounds [11–15]. They are biocompatible nanoparticles that are scalably processed through impact events, such as detonation [16]. Recently, a "clustered" nanodiamond drug delivery system that forms a porous cluster structure has been explored in biomedicine [17]. The unique structure enables functional features to the complex, such as control release and target therapy. NDs have also been widely explored in the imaging and labeling fields for biomedicine. Notably, NDs were functionalized to form photostable nanoprobes for optical imaging, modified to improve luminescence for biolabeling, and coupled with plant bioactive metabolites for cancer therapy [18–21]. Additional studies have also been conducted to explore NDs applications in bioimaging, drug delivery, and numerous other biomedical applications [22–27]. These characteristics enable the NDs to serve as a promising drug delivery system for enhancing chemotherapy efficacy.

In this study, Doxorubicin (DOX), an efficient chemotherapeutics, has been explored to be adsorbed and released from carboxylated nanodiamonds (COOH-NDs) and original nanodiamonds. The preparation conditions of NDs and DOX complexes (NDX) were explored from the aspect of ND/DOX ratio, DOX adding speed, pH, and temperature. The ideal drug releasing conditions of NDX were optimized as well. The release profiles of NDX complexes were quantified in temperature-dependent, pH-dependent, ion strength-dependent, and antibiotics competitive environments. The optimized NDX complex may enhance the delivered drug amount and extent release time at the desired location, and thus have the potential to greatly enhance the treatment of cancer

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2. Materials and methods

2.1. NDX synthesis and characterization

NDX complex was synthesized by diluting dispersible ND with autoclaved H_2O (XF Nano Materials Tech Co. Ltd., Nanjing, China) to generate 5 mg/mL ND solution. 2 mL of 2 mg/mL Doxorubicin (Adamas Reagent Ltd. Co., Shanghai, China) solution was then added to the ND solution at 20 μL/s using the syringe pump (Longer Pump Ltd. Co., Hebei, China) and mixed along with 0.1 mL of 0.1 M NaOH solution, which induced the formation of NDX aggregates for complex stability. The result NDX complex was sterilized in autoclave and ultra-sonicated (Scientz SB-5200D, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) in ice bath for 2 h before incubating in dark environment for 2 days. The pH of the NDX complex was measured using pH meter (Sartorius, Shanghai, China). After incubation, NDX complex was then centrifuged (Eppendorf 5427 R, Eppendorf Co. Ltd., Shanghai, China) for 10 min under 4 °C. The first resulting supernatant was saved for further UV–vis analysis, and the resulting NDX pellet was then further resuspended in H_2O and centrifuged for another 10 min. The second resulting was again saved for further UV–vis analysis, and the dark purple pellet was redispersed in H_2O. After dispersing the NDX complex, pH of the complex was measured again. The redispersed solution was then ultra-sonicated for 4 min to generate NDX solution. Total of 13 NDX complexes were synthesized with different ND/DOX ratio, NaOH concentration, and DOX adding speed. Refer to Table 1 for their respective compositions.

Doxorubicin was serial diluted into various known concentrations for the generation of a respective standard curve using UV–vis spectroscopy (AuCy Instrument, Shanghai, China). A linear relationship between absorbance and concentration was generated through serial dilutions. Two resulting supernatants from NDX synthesis were analyzed using UV–vis with wavelengths ranging from 400 to 500 nm. Their respective concentrations were calculated by using the measured absorbance and linear equation formulated through serial dilutions. The linear relationship was then used to quantify the amount of DOX desorption from NDs. Additionally, the characterization of NDX was performed using Zetasizer Nano for Dynamic Light Scattering (DLS) and ζ-Potential analyses (Malvern Inc., Shanghai, China). The solvent used in DLS analysis was deionized water. Refer to Fig. 1A for a summary of the synthesis and characterization of NDX complexes.

2.2. COOH-NDX synthesis and characterization

The dispersible ND powders were heated in a 9:1 (v/v) mixture of concentrated H_2SO_4 and HNO_3 at 75 °C for a day [37]. Subsequently, the ND solution was then heated in 0.1 M NaOH aqueous solution at 90 °C for 2 h, and later heated in 0.1 M HCl aqueous solution at 90 °C for 2 h. The oxidized and carboxylated ND (COOH-ND) was extensively rinsed with deionized water and centrifuged (Beckman Coulter Avanti J-26 XPI, Beckman Coulter Co., Ltd., Shanghai, China) three times at 20,000*g* RCF. The synthesis and characterization of COOH-NDX are identical to the methods used for NDX complexes. Refer to Table 1 for the compositions of COOH-NDX complexes.

2.3. NDX and COOH-NDX release profile

After characterizing NDX and COOH-NDX solutions, each complex was then divided into 2 tubes and kept in dark environment for further release profile quantification. NDX solutions were centrifuged for 20 min and all the resulting dark purple pellets were further dispersed with H_2O. Supernatants were saved for UV–vis analysis to quantify the release profile of NDX and COOH-NDX complexes. To determine the most optimized DOX release environment, each of the tubes containing NDX and COOH-NDX was kept in different environments. Specifically, some NDX were kept in room temperature, 37 °C, or acidic pH 5 environment. Additionally, some release profiles were quantified in environments that have either additional ions or antibiotics present (Ampicillin, Ciprofloxacin, and Tetracycline) (Thermo Fisher Scientific, Shanghai, China) that competitively bind to NDs. The aforementioned antibiotics (1 mg/mL), commonly used in combination with chemotherapeutics for cancer treatment, were quantified in both room temperature and 37 °C environments. The quantification of NDX and COOH-NDX release profile was monitored for 240 h and 192 h, respectively. Refer to Table 2 for the environments to which NDX and COOH-NDX complexes were kept for the quantification of DOX release profile and refer to Fig. 1A for a summary of the quantification of release profiles of the complexes.

3. Results

3.1. NDX & COOH-NDX synthesis

An illustration of NDX and COOH-NDX complexes is shown in Fig. 1B. After 2 days of incubation and multiple repeated centrifugations, Doxorubicin was successfully loaded onto NDs. The loading capacity in percentages and the amount of DOX loaded in weight are illustrated in Fig. 2A. The highest loading capacity and amount is observed in NDX#3 (2 mL ND, 5 mL DOX, 0.061 mL NaOH, and 20 μL/s DOX adding speed) in which the ratio of ND to DOX is 1:2.5. From this result, the difference in NaOH concentration and ND/DOX ratio affects the loading capacity of NDX complexes the most, while DOX adding speed and carboxylated NDX are insignificant in increasing loading capacity and amount. NDX complexes in which the amount of DOX added was greater than that of ND experienced greater loading capacity and amount. Moreover, NDX#5, with excess amount of NaOH, exhibited the lowest loading capacity and amount. Excessively induced aggregations resulted in the inhibition of drug binding onto the NDs.

3.2. NDX & COOH-NDX characterization

NDX loading capacity was further confirmed after the measured UV–vis intensities were implemented in a standard curve of DOX, as shown in Fig. 2B. The shifts in pH values immediately after synthesis and before the quantification of release profile are presented in Fig. 3A. NDX complexes shifted towards neutral and acidic during the synthesis

Table 1
Compositions of NDX complexes. Total of 13 different NDX complexes were synthesized to evaluate the optimal compositions and loading efficiency.

NDX synthesis													
Factors	ND/DOX ratio			NaOH concentration		DOX adding speed			COOH-NDX		Antibiotics-NDX		
ND	2 mL	4 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
DOX	2 mL	2 mL	5 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	5 mL	2 mL	2 mL	2 mL
NaOH	0.1 mL	0.15 mL	0.061 mL	0.061 mL	0.145 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.061 mL	0.1 mL	0.1 mL	0.1 mL
DOX speed	20 μL/s	20 μL/s	20 μL/s	20 μL/s	20 μL/s	5 μL/s	200 μL/s	500 μL/s	20 μL/s	20 μL/s	20 μL/s	20 μL/s	20 μL/s
NDX#	1	2	3	4	5	6	7	8	9	10	11	12	13