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Purification and functionalization of nanodiamond to serve as a platform for amoxicillin delivery

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ABSTRACT

Urinary tract infections (UTIs) cost $0.4–0.5 billion a year in the US and is the second most common disease affecting millions of people. As resistance to antibiotics becomes more common, a greater need for alternative treatments is needed. Nanodiamond particles (NDPs) are actively researched as drug delivery platforms due to their biocompatibility, particle size, and stable inert core. This research is aimed at developing NDPs as antibiotic drug delivery platforms for treating UTIs. To this end, 100 nm, 75 nm, 25 nm and 6 nm size NDPs are purified with acid and heat treatment techniques. Raman spectra of the NDPs showed that the acid treatment method resulted in higher diamond yield. Fourier transform infrared spectroscopy (FTIR) studies showed that both purification techniques result in oxygen terminated surface groups. Efficiency of loading amoxicillin on 25 nm NDPs based on electrostatic interaction of NDPs, functionalizing surfaces of NDPs with hydrogen, and polyethylenimine (PEI) are investigated. It is found that the electrostatic and surface hydrogenation approaches are not efficient in loading amoxicillin on the NDPs. On the other hand, PEI functionalized NDPs produced successful loading with amoxicillin as indicated by the presence of the β-lactam peak at 1770 cm^{-1}, amide peak at 1680 cm^{-1}, and bond between PEI NH stretching and amoxicillin –COOH group at 3650 cm^{-1} by the FTIR spectra. These results are expected to lay the foundation for developing NDP based targeted drug delivery treatment techniques for treating UTIs and other infectious diseases.

1. Introduction

Bacterial urinary tract infections (UTIs) are the second most commonly reported infection in the United States. Antibiotics such as amoxicillin are used to treat UTIs. However, development of bacterial resistance to antibiotic treatment still remains a major drawback for curing infectious diseases such as UTIs [1]. Also, in order to avoid side effects of antibiotics, alternate approaches such as targeted drug delivery are being actively developed. Nanoparticles are good candidates for drug delivery platforms because of their large surface area but have to satisfy certain criteria such as low toxicity profile, surface functionalization, biocompatibility, and maintenance of colloidal stability under physiological conditions [2,3]. Nanodiamond particles (NDPs) are emerging class of carbon nanomaterials, which offer versatile properties such as high thermal conductivity, electrical resistivity, thermal stability, [4–6] optical transparency, chemical inertness, high specific area, hardness, thermal stability, and excellent bio-compatibility [7,8]. It has been shown that NDPs are nontoxic to a variety of cell types such as neuroblastoma and kidney cells [9–12]. The low toxicity profile and high cellular uptake of NDPs make them well suited as a drug delivery platform. As a result, NDPs are now considered as a nontoxic alternative to semiconductor quantum dots for biomedical imaging, drug delivery, and other areas of medicine [13].

NDPs can be synthesized on a large scale by the detonation of explosive mixture of trinitrotoluene and hexogen in a closed chamber. Other synthetic methods include chemical vapor deposition and shock compression of graphite at high temperature and high pressure [14,15]. Processing of NDPs may utilize techniques such as ultra-sonication, centrifugation, and mechanical milling, which may lead to contamination that requires further purification. Acid treatment or air oxidation of NDPs not only removes impurities but also functionalizes the surface with carboxyl groups that can be used for binding drugs.

As of today, NDPs have been successfully evaluated as a vehicle for chemotherapeutic and protein based drugs [16–19]. NDPs functionalized with low toxicity polyethylenimine (PEI) have been used for DNA, siRNA [20] and gene delivery [21]. There are some studies on antibacterial properties of NDPs and menthol functionalized NDPs on gram negative and positive bacteria *Escherichia coli* [22,23]. Recently Lee et al. [24] studied loading of amoxicillin on non-functionalized NDPs embedded in Gutta Percha as a dental filler composite by mixing NDPs and amoxicillin for 5–7 days. They found that after mixing, the dynamic size of the NDPs increased by 100 nm and FTIR spectrum confirmed the presence of amoxicillin on the surface. However since there

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is no washing after mixing, it is not clear if there is conjugation of amoxicillin to the NDPs, and if the antibacterial effects are due to the unbonded (free) amoxicillin. We believe that in order to design a drug delivery platform based on NDPs, it is essential to establish conjugation between the amoxicillin and the NDPs and to consider the washing steps to remove the excess amoxicillin. To the best of our knowledge, there has been no study on loading the antibiotic amoxicillin on NDPs for treating UTIs. In this paper, we demonstrate the successful development of a technique for loading amoxicillin on NDPs.

2. Materials and methods

NDPs synthesized by detonation method with an average size of 6 nm were purchased from Nanostructured and Amorphous Materials Inc. Aqueous slurry of monocrystalline diamond particles (MDP) with average particle size of 25 nm, 75 nm, and 100 nm were purchased from Advanced Abrasive Corporation. All monocrystalline diamond particles were synthesized by high pressure high temperature method. H_2SO_4, HNO_3 and HCl were purchased from Fisher Scientific, (98.06% purity). NaOH was obtained from ACROS organics (99.00% purity). Polyethylenimine solution (50% w/v in water) was purchased from Fluka. Amoxicillin was obtained from Sigma Aldrich. Allegra X-30 Beckman Coulter centrifuge and Laurell Technology Corporation spin coater were used for centrifugation and spin coating experiments, respectively. NDPs were characterized using a Nicolet Almega XR Dispersive Raman 960 spectrometer with 532 nm laser excitation over the range 750–2500 cm^{-1} at 80% of incident laser power (Max power: 150 mW), Agilent Varian 680-IR Fourier Transform Infrared (FTIR) spectrometer with PIKE VeeMAX II reflection mode with an incident beam angle of 70, and Scanning Electron Microscopy (SEM, Hitachi S-4800).

2.1. NDP purification

Initially, 10 ml of MDP slurry was evaporated at 70 °C to obtain 25 nm, 75 nm, and 100 nm NDPs. The dried NDPs and the commercially purchased 6 nm NDPs were used as initial materials and are henceforth called "as received" NDPs. Two approaches were employed to purify the as received NDPs: heat treatment and acid treatment. In the heat treatment technique, NDPs of each size were heated separately at 500 °C for 5 h in air. Acid treatment (Ushizawa et al. [25]) involves refluxing the NDPs in a 9:1 (v/v) mixture of concentrated H_2SO_4 and HNO_3 at 70° for 24 h in which carbon impurities are removed, followed by 0.1 M NaOH aqueous solution at 70 °C for 2 h, which neutralize the surface from sulfuric and nitride ions, and finally in 0.1 M HCl aqueous solution at 90 °C for 2 h, in which metal impurities were removed. The resulting NDP suspension was washed with deionized water and separated by centrifugation at 13,000 rpm. The dried collected powder was prepared for Raman, FTIR and SEM characterization. Raman spectroscopy measurements were performed on 4 different locations on a given NDP sample in order to investigate the homogeneity. For FTIR characterization, 200 μl of deionized water was added to 1 mg of a given NDP sample. The entire suspension was added to an aluminum coated silicon substrate and dried. FTIR spectra were collected in the reflection mode with an incident beam angle of 70°.

It is to be noted here that the final goal of our experimentation was the loading of amoxicillin on purified NDPs. To achieve this goal, we have followed a careful experimental procedure wherein preliminary experiments were carried out using the "as received" suspensions to develop the appropriate processing techniques for loading amoxicillin on NDPs. Once amoxicillin loading was demonstrated, we replicated the results using the purified NDPs. Furthermore, it is to be noted that all our experiments were replicated multiple times in order to ensure that our results are consistent and reproducible. This systematic and careful experimental procedure resulted in successful demonstration of amoxicillin loading on purified NDPs.

2.2. Amoxicillin loading on NDPs

The following approaches were studied for loading amoxicillin on NDPs: 1) electrostatic interaction, 2) surface hydrogenation, and 3) surface functionalization of NDPs with PEI.

2.2.1. Electrostatic interaction

To develop a rapid and simple method, which does not increase toxicity and size of NDPs, electrostatic interaction between NDPs and amoxicillin was studied. We sought to change the surface charge of NDPs and amoxicillin by varying the pH, and engender amoxicillin loading through electrostatic interactions. Fig. 1 shows a schematic diagram of the experimental setup to measure the NDP charge. A voltage (5 V) was applied between two platinum electrodes dipped in a NDP slurry. The surface charge on the NDP was identified by observing which electrode surface was coated. HCl (0.01 M) and NaOH (0.01 M) were used to adjust the pH of NDPs and amoxicillin. The obtained NDPs (1–4 mg/ml) and amoxicillin (1–4 mg/ml) with opposite surface charges were mixed with a magnetic stirrer for 24 h, then centrifuged at 13,000 rpm followed by two steps of washing with DI water to remove the excess amoxicillin (c.f. row no. 1 of Table 3).

2.2.2. Surface hydrogenation

For experimental confirmation of surface hydrogenation, chemical vapor deposition (CVD) thin film diamonds were used as test-bed samples [26]. The CVD thin films and nanodiamond powders were simultaneously loaded into a microwave plasma chemical vapor deposition system (MPCVD) and exposed to hydrogen plasma (pressure 60 Torr, temperature 800 °C and microwave power 3.49 kW). Surface charge of the resultant NDPs was measured by the method illustrated in Fig. 1. Hydrogenated NDPs were mixed with amoxicillin with the opposite surface charge as described in previous section (c.f. row no. 2 of Table 3).

2.2.3. Surface functionalization of NDPs with PEI

To develop an alternative approach for functionalizing the NDP surface, polyethylenimine (PEI) was chosen due to its low toxic profile and previous use for successfully conjugating NDPs with DNA and siRNA [20]. Due to ease of handling and characterization, initial experiments

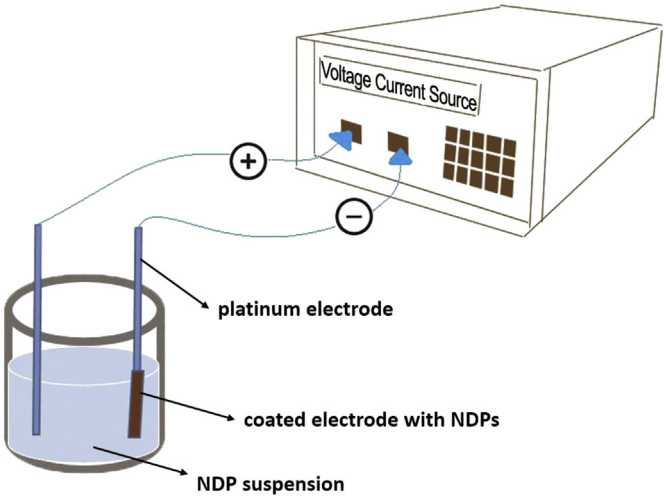

Fig. 1. Schematic diagram of experimental setup used to measure the surface charge of NDPs. Two platinum electrodes were connected to voltage source (applied voltage 5 V). NDPs were mixed in deionized water and the pH was adjusted to the desired value. The surface charge of the NDPs was inferred from noting the electrode on which a coating of NDPs was observed. The NDPs have charge opposite to the polarity of the coated electrode.