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Sodium alginate-functionalized nanodiamonds as sustained chemotherapeutic drug-release vectors

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ABSTRACT

Prolonged therapeutic action over an extended period of time on single dose is vital in designing an ideal drug delivery system. Nanoparticle-based vehicles for drug delivery serves as an ideal technology, however, utilization of them remains controversial due to potential safety concerns and transient drug retention. Nanodiamonds (NDs), with excellent biocompatibility and diversity of potential conjugates, have emerged as alternative promising materials in this regard. Here, we developed sodium alginate functionalized NDs (fNDs) for drug delivery. Cisplatin (DDP) was chosen as a model drug and coated on the negatively charged fNDs clusters. Our work demonstrated that fNDs were capable of enhancing drug accumulation and retention time in tumor cells, leading to continued tumor cell killing effect after clearance of drug treatment. Furthermore, fNDs–DDP treatment improved drug safety compared to DDP treatment. Thus, this new platform may have strong potential for further biomedical application.

1. Introduction

Prolonged therapeutic action over an extended period of time on single dose is an important issue in engineering an ideal drug delivery system for cancer therapy [1]. On one hand, it can avoid the repeated administration to reduce the suffering of patients. More importantly, it will reduce of the overall quantity of drug used, resulting in fewer side effects that can significantly complicate the course of treatment. Additionally, it will also reduce the cost of treatment.

Utilization of nanoparticle-based vehicles for chemotherapeutic delivery serve as an ideal technology, as their advantages include small size induces permeability changes in cell membranes, enhancing cellular uptake, and strong ability to load large amounts of drugs, leading to the reduction of the overall quantity of drug used [2]. The family of nanocarriers include polymer, nano-liposome, carbon nanomaterials, and gold nanoparticles [3–7], while some of them still have safety concerns [8–11]. In addition, the intracellular retention time of drugs loaded by these nanomaterials is transient [12–16], which harmers their translation into further biomedical application.

Nanodiamonds, 2–10 nm diameter carbon carriers of truncated octahedral composition, have emerged as alternative promising materials attracting considerable research interests in this regard. They possess excellent biocompatibility [17–20] and diversity of potential conjugates [21,22]. Literature and our previous studies of pristine NDs have demonstrated their carriers abilities with metal ions and small molecule drugs with increased biological outcomes [23–27]. In this work, we develop polysaccharide sodium alginate (ALG) functionalized NDs (fNDs) for chemotherapeutic drug delivery. *Cis*-diamminedichloro platinum(II) (Cisplatin, DDP), one of the most potent antitumor agents [28], was coated on the negatively charged fNDs clusters and introduced into various kinds of tumor cells. The resulting fNDs-based drug delivery system significantly improved drug accumulation and retention in tumor cells, which led to continued tumor cell killing effect after clearance of drug treatment (Fig. 1). Additionally, fNDs–DDP treatment improved drug safety compared to DDP treatment. As such, this new fNDs-based delivery system is a promising drug delivery platform for further application.

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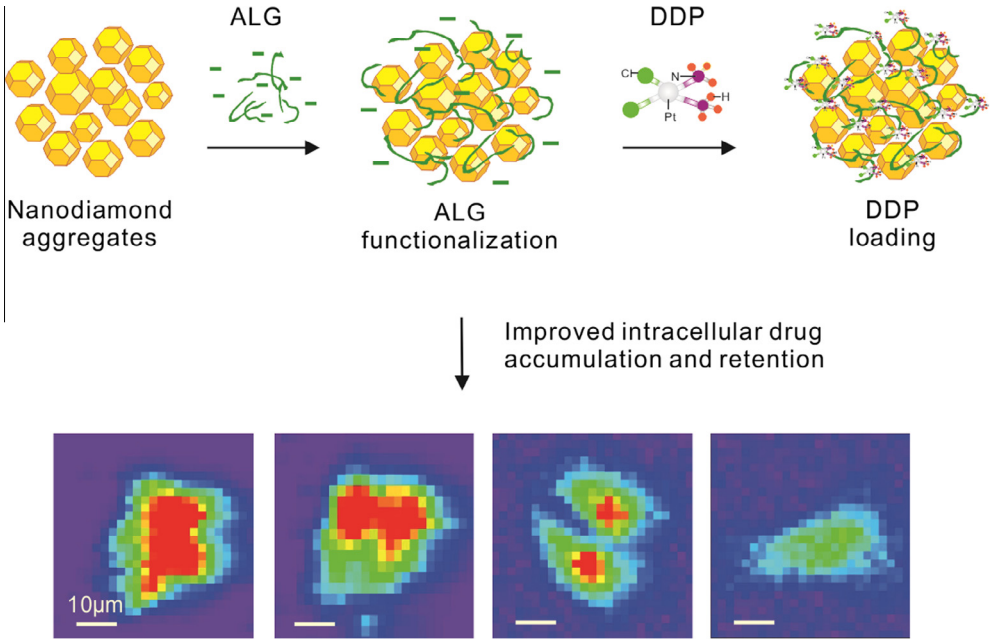

Fig. 1. Schematic showing of sodium alginate functionalized NDs (fNDs) improved drug accumulation and retention in tumor cells.

2. Materials and methods

2.1. Materials

NDs with individual sizes of 2–10 nm synthesized by detonation techniques were supplied by Gansu Gold Stone Nano. Material. Co. Ltd., China. When dispersed in aqueous solution, they can spontaneously form clusters of tens to hundreds nanometers with a lower free energy [20]. The details for characterization have been described in our previous work [27].

Cisplatin (DDP) were obtained from Sigma–Aldrich, USA. Stock solution of DDP was prepared with distilled millipore water and was used to make serial dilutions. All other chemicals used were of analytical grade.

2.2. Preparation of fNDs–DDP

To prepare NDs-ALG (fNDs), NDs (2 mg) were sonicated in sodium alginate (ALG, Sigma–Aldrich) solution (4 mg in 0.1 M aqueous NaCl, 4 mL) for 20 min and then stirred at room temperature for 24 h. The fNDs complexes were isolated by centrifugation at 13,000 rpm for 20 min, and then washed with millipore water in more than five washing/centrifugation cycles to remove the excess ALG.

To prepare fNDs–DDP, DDP was stirred with fNDs at a w/w ratio of 2:1 in millipore water for 24 h at room temperature, the fNDs–DDP complexes were isolated by centrifugation at 13,000 rpm for 20 min, and then washed with millipore water in more than five washing/centrifugation cycles to remove the excess DDP. The amount of unbound DDP was determined by measuring the Pt concentration in all of the supernatants by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Arcos Sop, Spectro, Germany), allowing the drug loading efficiency to be estimated.

The apparent hydrodynamic size and zeta potential of NDs, fNDs and fNDs–DDP were measured by laser light scattering with a Delsa NanoC particles Analyzer (BECKMAN COULTER, America). The concentration of the samples was 20 μg/mL.

2.3. Cell culture

HepG2 liver carcinoma cells and bronchial epithelial BEAS-2B cells were grown in RPMI1640 (Gibco) cell culture medium supplemented with 10% fetal bovine serum (FBS). Cervical cancer HeLa cells, lung cancer A549 cells and RAW264.7 macrophages were grown in DMEM (Gibco) cell culture medium supplemented with 10% FBS.

2.4. Synchrotron-based micro X-ray fluorescence (μXRF) microscopy

The Mylar X-ray films (Hoffman, 12 μm thickness) were previously put into 24-well plates and sterilized by successive baths in 70% ethanol. HepG2 cell suspension (6×10^4 cells/mL) was dispensed into 24-well plates and incubated overnight to allow for cell adherence to the thin films. fNDs–DDP (50 μg/mL), fNDs (50 μg/mL), and DDP (3.3 μg/mL) were added into the plate wells, respectively. After 6 h incubation, cells were washed with PBS to remove the uninternalized nanoparticles or drugs. Then, cells were further incubated in cell culture medium for 72 h and washed every 24 h. μXRF microscopy was performed at 0 (denoted by 6 h in text), 24, 48, and 72 h after initial 6 h incubation at the beamline BL15U1 of SSRF. At each time point, cells were fixed with a few of 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS). After wash with PBS, the cells were then dehydrated in a graded gradient ethanol series and dried under air for X-ray imaging.

Incident X-rays energy of 14 keV, obtained with a Si (111) monochromator, was chosen in order to excite the L-lines of X-ray fluorescence of Pt element (Fig. S1). A light microscope was coupled to a computer for sample viewing and the sample platform was moved by a motorized X-ray mapping stage. A Kirkpatrick–Baez mirror system focused the X-ray beam to a spot size of 2 μm × 2 μm on the specimen, which was raster-scanned. XRF from the specimen was captured with an energy dispersive silicon drift detector (Vortex, USA). From the analysis of the X-ray fluorescence spectrum for each pixel, a spatial image can be obtained for each element separately. Such an image represents a two-dimensional projection of the volumetric distribution of the