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Nanodiamond-conjugated transferrin as chemotherapeutic drug delivery

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ABSTRACT

Targeted therapy is considered to be a promising strategy of cancer treatments. Herein we demonstrate the potential of a nanotechnology strategy for special drug delivery based on nanodiamond (ND) functionalized by transferrin. Specifically, the transferrin–doxorubicin (Tf–DOX) complex is covalently coupled with carboxylated ND to prepare the targeted nanomedicine (ND-(Tf–DOX)). *In vitro* studies showed that ND-(Tf–DOX) can effectively deliver the drug inside living cells via a clathrin-dependent and transferrin receptor-mediate endocytosis pathway by flow cytometry analysis, and the ND-(Tf–DOX) nanomedicine located in the lysosomes through laser scanning confocal microscopy. *In vivo*, we found that tumor volume change of tumor-bearing mice treated with ND-(Tf–DOX) nanomedicine was lightly lower than that of DOX-treated. The results demonstrated that the targeting capability of ND-(Tf–DOX) nanomedicine relative drug alone is not significant, and it may be disturbed by proteins when they are placed in a complex biological environment. However, it can markedly decrease side effects compared to DOX alone in both the liver and spleen. This suggests that *in vivo* ND-(Tf–DOX) nanomedicine can effectively inhibit tumor growth as the same as DOX. More importantly, it can decrease side effects. This study attempts to endowed transferrin-functionalized and chemotherapeutic drug-loaded nanodiamond as a platform for drug delivery and therapy to cancer cells.

1. Introduction

Recently, a promising biomaterial with tremendous potential for gene, protein and drug delivery has been placed on diamond-based platforms including ND produced via detonation and high pressure–high temperature (HPHT), ultra-/nanocrystalline diamond films (UNCD/NCD), single/polycrystalline diamond films, synthetic type Ib nanodiamond (ND) powders owing to their low toxicity, high chemical stability, high affinity to biomolecules, and ease of surface functionalizations [1–5]. Besides, fluorescent nanodiamond (FND) acted as optical bioimaging applications and drug delivery carrier has also attracted strong interest, which was produced by ion irradiation and subsequent annealing of synthetic type Ib nanodiamond powders, since FND with negatively charged nitrogen vacancy (NV^-) defect centers shows no signs of photobleaching even after its surface is functionalized with various biomolecules [6–9]. In the context of biological and medical applications, several biocompatibility studies of the nanodiamond particles *in vitro* or *in vivo* have shown that nanodiamond particles are among the least toxic of all carbon-based nanomaterials tested so far [10,11].

Many chemotherapeutic molecules possess a generalized mechanism of activity so that they act on both healthy and cancer cells. It is well known that doxorubicin (DOX) is an effective and widely used cancer chemotherapeutic agent. However, its clinical application is limited by dose-related toxic side effects, including cumulative cardiotoxicity and myelosuppression. Hence, a critical challenge is the ability to deliver sufficient amount of drug to the desired location with fewer side effects than that of conventional therapies. With the development of nanotechnology, the strategy of the nanoparticles as carriers for drug to protect against cytotoxicity for healthy cells has attracted wide attention. The studies of functionalized nanoparticles with cellular targeting elements (e.g., antibodies, aptamers, and ligand) would add another level of intelligence to the carrier technology to drive targeted drug delivery [12–15]. For instance, transferrin (Tf) has been exploited as an important ligand for specific labeling of cancer cells because of its ability to react with the membrane-anchored transferrin receptors (TfRs), which can be actively expressed on the surface of various tumor cells [16]. The interaction of transferrin and transferrin-receptor has therefore been acted as a potential efficient pathway for the cellular uptake of drugs and genes [17–19]. Based on the strategies, an attractive formulation for specific cellular uptake and targeted drug delivery was designed by Tf-functionalized nanomaterials [20–24], where the nanoparticles including quantum dots/rods [25], gold nanoparticles and nanodiamond were conjugated with Tf for imaging [26,27], and cancer diagnosis and biological labeling applications have been emerged as a promising class of tools. It is worth noting that the most promising and exciting applications of such nanomaterials involving in their potential utilization of Tf-functionalized [20–25], in contrast, little information has been

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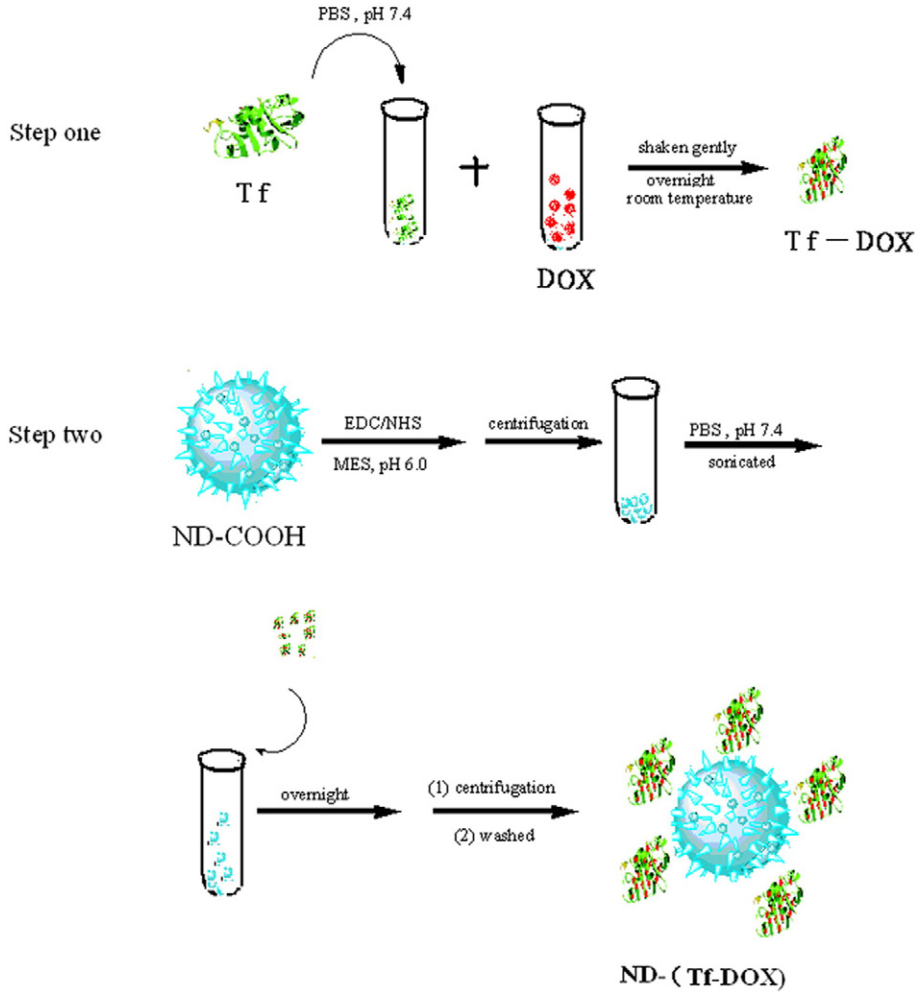
seen about transferrin-conjugated nanoparticles indeed delivery drug for cancer cells therapy. However, targeted therapy of cancer cells is exciting and the most important, rather than targeting the vector preparation alone. Herein we will demonstrate the potential of a nanotechnology strategy in cancer treatment.

In the work, we present that the carboxylated nanodiamond was conjugated with the Tf–DOX complex to form ND-(Tf–DOX) nanomedicine for targeted therapy of cancer cells. It is an extension of our previous studies of ND-DOX/ND-Tf *in vitro* anti-cancer efficacy [9,27]. Furthermore, it is also a deeper research on our previous report in transferrin-conjugated nanodiamond as an intracellular transporter of chemotherapeutic drug and targeting therapy for cancer cells [26], where the PEGylated FND conjugated with Tf (FND-PEG-Tf) as a drug delivery carrier, DOX is physically adsorbed onto the FND-PEG-Tf to obtain FND-PEG-Tf–DOX nanoparticles, and the amount of DOX adsorbed is only 10 μg/mg. In addition, unfortunately, only its effects on cell activity *in vitro* were discussed. The drug loaded is low so that more materials have to be used to achieve a therapeutic efficacy. However, long-term effects caused by the material remain a problem *in vivo* before clinical use. In order to solve this drawback, we designed a new drug system to study in detail their biological effects *in vitro* and *in vivo*. In view of these observations, we were interested to know, for instance, can they be internalized by cells as well? If so, what means do they enter the cell by? Whether their endocytosis pathway was similar to that of the transferrin themselves? In addition, what is the function of the ND-(Tf–DOX) nanomedicine *in vivo* with a complex environment? With these questions in mind, the efficacy of ND-(Tf–DOX) nanomedicine *in vitro* and *in vivo* was carried out.

2. Materials and methods

2.1. Materials and measurements

Synthetic type Ib nanodiamond powders (mean sizes of 100 nm) were obtained from Element Six. Human transferrin was bought from Sigma. Doxorubicin hydrochloride (DOX) was purchased from Shenzhen Main Luck Pharmaceuticals Inc. (China). Coomassie brilliant blue G-250 (CBB G-250) was given as a gift. Lyso-Tracker Red was purchased from Sigma. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Solarbio. HepG2 cells were provided by the Gene Engineering Center of Shanxi University. Dulbecco Minimum Essential Media (DMEM) was purchased from Thermo Fisher Biological and Chemical Product (Beijing, China). Fetal bovine serum was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. Trypsin was purchased from Sino-American Biotechnology Company. EDTA and 4% paraformaldehyde were obtained from Solarbio. BisBenzimide (Hoechst 33258, blue fluorescent dye) for staining nuclei was purchased from Beyotime Biotechnology, China. Millipore filtered water was used for all aqueous solutions. The list of equipments and instruments used is as follows: water-jacketed CO_2 cell incubator (Shanghai Lishen Scientific Instruments Co., Ltd), FQ-100DE numerical control ultrasonic wave washing machine (Kunshan Ultrasonic Instrument Co., L), laser scanning



Scheme 1. ND-(Tf–DOX) nanoparticle fabrication process.