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The tumor-targeting core–shell structured DTX-loaded PLGA@Au nanoparticles for chemo-photothermal therapy and X-ray imaging

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ABSTRACT

In this study, an organic–inorganic hybrid nanocomposite was synthesized by deposition of Au onto the surface of docetaxel (DTX)-loaded poly (lactide-co-glycolide) (PLGA) nanoparticle cores to form the core–shell structured DTX-loaded PLGA@Au nanoparticles. The tumor targeting peptide, angiopep-2, was then introduced onto the gold nanoshell through Au–S bond, achieving drug delivery with active targeting capability. This novel system allowed combined chemotherapy and thermal therapy for cancer, resulting from DTX and gold nanoshell. The formation of tumor-targeting gold nanoshell surrounding PLGA nanocore, designated as ANG/GS/PLGA/DTX NPs, was confirmed by its surface plasmon resonance (SPR) band in the UV–Vis spectrum and by a transmission electron microscope (TEM). The release profiles of DTX from this system showed strong dependence on near-infrared (NIR) laser. Compared with DTX alone, the ANG/GS/PLGA/DTX NPs afforded much higher anti-tumor efficiency without obvious toxic effects. Besides, it also showed potential X-ray imaging ability. These results demonstrated that the tumor-targeting core–shell structured DTX-loaded PLGA@Au nanoparticles could be used as a multifunctional nanomaterial system with NIR-triggered drug-releasing properties for tumor-targeted chemo-photothermal therapy and theranostics.

1. Introduction

Chemotherapy is still the mainstay for cancer therapy. Docetaxel (DTX), a microtubule-stabilizing taxane, was commonly used to treat systemic cancers. However, it still has some shortcomings, such as poor solubility in water, non-specific targeting and bone marrow suppression, which significantly limit their clinical applications [1]. In order to overcome these limitations, successful preparation and better anti-cancer effect of DTX-loaded nanoparticles have been reported [2]. However, due to the multilevel complexities and variability, monotherapy relying on chemotherapy failed to cure cancer and cancer remains one of the most challenging health problems to human being [3].

Gold nanoparticles (Au NPs) are attractive photo-thermal agents for cancer therapy because they show efficient local heating upon excitation of surface plasmon oscillations [4–9]. Of particular interest is the gold nanoshell-based platform due to its strong absorbance of NIR light and higher heat conversion efficiency [10]. In order to enhance the anti-tumor effect, the combination of photo-thermal therapy based on gold nanoshell and chemotherapeutic agents is particularly a promising strategy for optimizing cancer therapy [11]. However, the reported gold nanoshells are usually made up of an inner core and a continuous gold surface, which forms a barrier for release of the delivered drugs in the inner core [12]. To explore the combination therapy, Liang et al. [13] reported that 'smart' gold nanoshells can carry a drug payload on the surface, and that their intrinsic near-infrared (NIR) plasmon resonance enabled the combination of chemotherapy and hyperthermia therapy. Therefore, to develop the well-designed gold nanoshells with an inner core capable of delivering drug and a non-continuous gold surface for photo-thermal therapy may be a new strategy for improving the drug-loading efficiency instead of drug grafting on the surface as described before. Organic–inorganic hybrid nanomaterials hold promise of molecular tune ability in conjunction with dimension-dependent properties, which may otherwise not be materialized by using either inorganic or organic nanoparticles independently. Gold nanoshells made up of organic–inorganic hybrid nanomaterial are the particularly encouraging drug delivery systems for combination of heat and chemotherapeutic agents.

Considering the FDA approved poly (lactic-co-glycolic acid) (PLGA) as an organic material with distinguished biocompatibility and biodegradability [14], golden PLGA nanoshells for combined thermotherapy and chemotherapy have become the focus of intensive research. Yoo et al. [15] studied a novel drug delivery system consisting of a poly(lactic-co-glycolic acid) (PLGA) matrix containing doxorubicin (DOX) as a chemotherapeutic agent and a gold over-layer on the polymer matrix capable of the photo-thermal effect. However, the *in vivo* application of gold nanoshells for combination therapy of heat and

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chemotherapeutic agents, particularly with tumor-targeting and optimized NIR irradiation to achieve combination effect in animals after systemic administration, remains to be explored.

Cancer detection with X-ray contrast agent is of great importance in clinic. However, present X-ray contrast agents are mainly based on iodine containing molecules, which are effective in absorbing X-ray but nonspecifically targeted with a very short imaging time window. Therefore, there is an urgent need to develop a new X-ray contrast agent with outstanding biocompatibility and strong X-ray attenuation. Recently, gold nanoparticles or nanomaterials containing Au have been developed for X-ray imaging [16,17]. Wen et al. [18] constructed multifunctional dendrimer-entrapped gold nanoparticles for dual mode CT/MR imaging applications. It was reported that the total numbers of gold per unit volume have an important role for X-ray imaging [19]. Compared with the solid gold nanoparticles, gold nanoshell-based nanoparticles have less gold content per unit volume. Thus, it is necessary to explore whether gold nanoshells have the potential for X-ray imaging.

In current study, the tumor-targeting core–shell structured DTX-loaded PLGA@Au nanoparticles were fabricated, which are designated as ANG/GS/PLGA/DTX NPs. This well-designed drug delivery system was constructed *via* an adapted emulsion solvent evaporation method for preparing the DTX-loaded PLGA NPs, followed by the formation of gold nanoshells by using a surface seeding method [20]. The presence of the glioma-targeting peptide, angiopep-2, on the gold nanoshell surface allowed the nanoparticles to offer active glioma-targeting ability along with passive targeting due to the enhanced permeability and retention. The ANG/GS/PLGA/DTX NPs were investigated by UV–Vis absorption spectroscopy, dynamic light scattering (DLS), zeta potential measurements and transmission electron microscopy (TEM). Interestingly, the release profiles of DTX from this system showed strong dependence on near-infrared (NIR) laser. Next, the anti-glioma effect of this system was evaluated in vitro and *in vivo*, respectively. The results demonstrated that the enhanced anti-glioma effect was obtained after combination of chemotherapy and phototherapy. Furthermore, *in vivo* X-ray imaging of tumor-bearing mice is also investigated. Thus, the novel drug delivery system developed in this work may be a promising multi-functional nanoplatform for cancer theranostic applications.

2. Materials and methods

2.1. Materials

PLGA (lactide/glycolide = 50:50, MW: 17,000 Da) was purchased from Jinan Daigang Biomaterial Co., Ltd. (Jinan, China). Poloxamer 188 was purchased from BASF Corporation of Germany (Local Agent in Shanghai, China). Docetaxel (DTX, purified >98%) was purchased from Dalian Meilun Biotech. Co., Ltd. (Tianjin, China). Polyethyleneimine (PEI, 25 kD), hydrogen tetra-chloroaurate (III) hydrate ($HAuCl_4 \cdot 4H_2O$), ascorbic acid (AA), and sodium borohydride ($NaBH_4$) were obtained from Aladin Ltd. (Shanghai, China). IR780 was purchased from Sigma-Aldrich, Inc. (St Louis, MO, USA). The water used was pretreated with the Milli-Q Plus System (Millipore Corporation, Bedford, USA). All other chemicals were of analytical grade and used without further purification.

All animals were obtained from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). All animal experiments were performed in accordance with the guidelines evaluated and approved by the Ethics Committee of Zhengzhou University.

2.2. Preparation of ANG/GS/PLGA/DTX NPs

The DTX-loaded nanoparticles (PLGA/DTX NPs) were firstly fabricated by a modified solvent evaporation method [21]. In brief, poloxamer 188 was dissolved in deionized water as the water phase at a concentration of 10 mg/mL. Organic phase was prepared by dissolving PLGA and DTX in acetone solution at a concentration of 10 and 1.5 mg/mL, respectively. The mixture was stirred at room temperature at 540 rpm for 4 h in order to ensure complete evaporation of the acetone. After that, PEI (25 kD) solution (10 mg/mL) was added to the PLGA/DTX NPs and the mixed solution was stirred for 4 h to prepare the PEI-modified nanoparticles.

Fig. 1 illustrated the procedure of fabricating the tumor-targeting core–shell structured DTX-loaded PLGA@Au nanoparticles. The GS/PLGA/DTX NPs were generated through two steps. The small gold nanoseeds were firstly formed on the surface of PEI-modified nanoparticles by reducing hydrogen tetrachloroaurate hydrate (100 mM) with sodium borohydride (10 mM), which resulted in the formation of a light red solution (GP/PLGA/DTX NPs). Subsequently, the gold nanoshells over the surface of PLGA NPs were constructed by slow growth of gold on the prior formed small nanoseeds by reducing hydrogen tetrachloroaurate hydrate (100 mM) with ascorbic acid (7.8 mM), followed by vigorously mixing for 2 min. The solution appeared purple red in color. Then the nanoparticles (GS/PLGA/DTX NPs) were collected by centrifugation at 12,000 rpm at 4 °C for 30 min.

An aliquot of 2 mL GS/PLGA/DTX NP solution (2 mg/mL) was mixed with 0.5 mg angiopep-2 and 1 mg HS-PEG_{2000}, and the mixed solution was reacted for 24 h at room temperature under nitrogen atmosphere. At last, the obtained ANG/GS/PLGA/DTX NPs were centrifuged, dispersed in PBS (pH = 7.4), and stored at 4 °C for further use. IR780-loaded PLGA NPs for *in vivo* studies were similarly prepared, except that DTX was replaced with IR780 in the formulation.

2.3. Nanoparticle characterization

DLS (Zetasizer Nano ZS-90, Malvern, UK) was used for characterizing nanoparticle size, polydispersity index and zeta potential of the nanoparticles. The morphology of PLGA/DTX NPs and ANG/GS/PLGA/DTX NPs was observed by TEM. The corresponding energy dispersive X-ray spectroscopy (EDX) was also performed to verify the elements of the prepared nanoparticles. The absorption spectra of PLGA/DTX NP, GP/PLGA/DTX NPs and ANG/GS/PLGA/DTX NPs were obtained by using an UV–Vis spectrometer (Shimadzu, Tokyo, Japan).

2.4. Determination of encapsulation efficiency and in vitro release study

The drug encapsulation efficiency was expressed as the percentage of entrapped drug with respect to the total amount of drug added. The DTX encapsulation efficiency in the DTX-loaded PLGA NPs was investigated as previously reported [22]. An HPLC (Agilent 1200, USA) equipped with a reverse-phase Intertex C18 column (Atlantis, 5 μm, 4.6 × 250 mm) and an UV–Vis detector was used. The mobile phase consisted of methanol:water (73:27). The eluent was monitored at 230 nm with a flow rate of 1 mL/min.

In vitro release of DTX from nanoparticles was performed in phosphate buffer saline (PBS) containing 1% tween-80 at 37 °C. In brief, 1 mL of nanoparticle suspension with a final concentration of 1.5 mg/mL of DTX was loaded into a dialysis tubing (cutoff mass 12 kD). The dialysis tubing was maintained in 40 mL PBS containing 1% Tween-80 and agitated (100 rpm) at 37 °C. A sample of 2 mL was taken from the release medium at the predetermined time points, followed by supplementation of a same volume of fresh medium into the system. The concentration of DTX in each sample was determined by HPLC after dilution with methanol. The HPLC assay was conducted as described above.

2.5. Measurement of temperature under NIR irradiation

ANG/GS/PLGA/DTX NPs at the concentration of 0.1 mg/mL and 0.5 mg/mL, respectively, were irradiated by an 808 nm laser at 2.5 W/cm^2. Temperature was measured at 30 second intervals with an infrared thermometer (S-211, Guangxi, China) placed inside the solution for a total of 5 min. The thermometer was not in the path of the laser