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"US-detonated nano bombs" facilitate targeting treatment of resistant breast cancer

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ABSTRACT

Reversal of drug resistance and targeted therapy are the keys but remain challenging in resistant breast cancer treatment. Herein, low frequency ultrasound detonated "nano bombs" were rationally designed and used for treatment of resistant breast cancer. For the 'nano bombs', the ammunition (Doxorubicin, DOX) was loaded into the ammunition depot (hollow mesoporous TiO_2, MTNs), and the safety device (dsDNA) was wrapped on the surface of MTNs to avoid the unexpected DOX release. We found the "US-detonated explosive" abilities of "nano bomb" MTNs (NBMTNs), including explosive generation of ROS, explosive release of DOX, US-triggered lysosome escape and mitochondrial targeting in the in vitro and in vivo studies. More importantly, the drug resistance of MCF-7/ADR cells could be reversed via the inhibition of mitochondrial energy supply approach caused by the "explosion" of NBMTNs. Furthermore, NBMTNs combined the superior chemotherapy efficacy of DOX and potent SDT efficacy in one single platform and significantly enhanced the anticancer efficacy. Our results demonstrate an approach for reversing resistance and specific targeting of tumors using 'US-detonated nano bombs'.

1. Introduction

There have been many efforts to conquer cancer with current chemotherapy. However, most of the cytotoxic chemotherapeutic agents are limited in their clinical applications due to the serious toxicities in patients, such as diarrhea, sickness, hair loss, myelosuppression, and cardio toxicity. In order to reduce the clinically used drug dosage and side effects, drug-delivery systems (DDS) have been developed to overcome this problem because they offered excellent efficient drug transportation [1]. In the past 10 years, nanoscaled drug delivery systems such as liposome, solid lipid nanoparticles (SLN), polymer micelles, mesoporous silica, carbon nanomaterial, and gold nanomaterial. etc., which increased tumor selectivity and reduced toxicity have been receiving a lot of attentions [2]. Although the above DDSs have their own advantages, many of the reported drug delivery systems still need to improve. For example, the drug release profile in the above DDSs is still a problem, most of the above DDSs do not allow for controlled release, the drug encapsulated in the DDS could not rapidly release in tumor, moreover, most of the DDSs always suffer from the unexpected drug release during circulations [3]. Therefore, to maximize the antitumor efficacy and minimize the side effect in cancer therapy, the one-demand drug delivery system with high spatial/temporal resolution in vivo is still needed.

Recently, stimuli-responsive drug delivery systems, such as pH-responsive, GSH-responsive, temperature-responsive, light-responsive bring many benefits to cancer treatment [4]. Among these DDSs, light-responsive DDSs have attracted more and more attentions due to their accurate positioning and control capabilities. However, the UV or visible light could not penetrate deeply and also could cause the damage to skin in vivo. In contrast, ultrasound (US) could penetrate deeply in the human bodies, even the internal organs, such as liver, spleen, kidney etc. [5]. In addition, ultrasound therapy has been used clinically for many years, its safety and effect had been clinically confirmed [6]. The high tissue penetrating ability and safety making ultrasound has a great promise for in vivo one-demand US-controlled drug delivery system [7,8]. Recently, titanium dioxide (TiO_2), a typical semiconductor material, has been widely used in many fields including biomedicine [9,10]. According to the previous studies, TiO_2 nanoparticles with little cytotoxicity could strongly absorb ultraviolet (UV) light or ultrasound (US) and generate a lot of reactive oxygen species

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(ROS, such as hydrogen peroxide, hydroxyl radicals, and super oxides) [11–13]. In this study, TiO_2 nanoparticles were chosen for the US-triggered drug delivery system.

Despite the advantages of DDSs in delivering chemotherapeutic agents, there is another serious problem usually occurs during the chemotherapy, multiple-drug resistance (MDR) [14]. The emergence of MDR is a main obstacle for successful treatment of cancer. Cancer cells often develop drug resistance and stop responding to chemotherapeutics after repeated sessions of chemotherapy. Generally, MDR is typically mediated by the overexpression of a membrane transporter, P-glycoprotein (P-gp), actively increases the efflux of drugs from cancer cells [15]. The efflux drugs reduced the therapeutic effect and cancer cells often develop drug resistance and stop responding to chemotherapeutics after repeated sessions of chemotherapy [16]. Therefore MDR is also a main obstacle for drug delivery systems. To overcome the obstacle, an idea drug delivery system should not only have the controlled drug release ability, but also promote the intracellular accumulation of drug in the targeted cells and maintain drug concentration in an optimum level to overcome the resistance pathways. One strategy to resolve this problem is to develop combining multiple conventional cancer treatment modalities in one system. For example, chemotherapy and photodynamic therapy (PDT) can potentially overcome drug resistance through different mechanisms of actions to achieve enhanced anticancer efficacy [17]. However, the short board of PDT is the light source, so in this study, to overcome this problem, a sonodynamic therapy (SDT) combined with a controlled chemotherapy were used to tumor-targeting drug delivery, tumor site-specific drug release and overcome drug resistance.

Herein, we rationally designed a US-detonated 'nano bomb' base on hollow mesoporous TiO_2 (MTNs) as the antitumor drug delivery system for drug resistance cancer treatment. As schemed in Fig. 1, the 'nano bomb' mainly includes 3 parts: the "ammunition" is doxorubicin (DOX), and the DOX is loaded in the "ammunition depot" (MTNs), to avoid unexpected DOX release, a "safety device" (DNA) is wrapped on the surface of MTNs for blocking the DOX release. The designed 'nano bomb' also has multiple benefits for drug resistance cancer treatment: (1) 'US-detonated explosive' generation of ROS, for tumor-specific SDT; (2) 'US-detonated explosive' release of DOX, for tumor-specific chemotherapy; (3) combined SDT and chemotherapy in one drug delivery platform, for efficient reversal of drug resistance; (4) EPR effect is optimized by a particle size control. The enhanced antitumor efficacy and "US-detonated" ability of the 'nano bomb' were examined using MCF-7/ADR cells and drug-resistant breast tumor models.

2. Experimental section

2.1. Materials

Doxorubicin (DOX, 20160511, purity > 98%) was gotten from Beijing Yi-He Biotech Co. Ltd. Titanium (IV) isopropoxide (TIP) (95%) was obtained from Alfa Aesar (Lancs, UK). Pluronic F127, N-(4-Maleimidobutyryloxy) succinimide (GMBS), IR 783 dye, (3-aminopropyl) triethoxysilane (APTES), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich Co. LLC. PCR primer, loading Buffer, and Golden View was obtained from Beijing Ding Guo Chang Sheng Biotechnology Co. Ltd. Gel Extraction Kit, 2 × Taq Master Mix, and 50 × TAE was obtained from Beijing Com Win Biotech Co. Ltd. Sulforhodamine B (SRB), DMEM cell culture medium, penicillin, streptomycin, fetal bovine serum (FBS), and heparin sodium were bought from Gibco Invitrogen. SOSG, DAPI, DCFH-DA, hematoxylin and eosin were supplied by Beyotime Biotechnology Co. Ltd. Other reagents were acquired from China National Medicine Corporation Ltd. Ultrasonic physiotherapy instrument: Chattanooga group 4714 Adams Road Hixson, TN 37343, power density: 0-10 W, probes area: 2 or 5 cm^2.

2.2. Synthesis of "Nano bomb- like" hollow mesoporous TiO_2 (NBMTNs)

2.2.1. Synthesis of hollow mesoporous TiO_2 (MTNs)

MTN nanoparticles were synthesized via a sol–gel route. Pluronic F127 was used as a structure-directing agent and TIP as a TiO_2 source. In detail, 6 g F127 was completely dissolved in 250 ml ethanol, and then 1 mL water was added to this solution with stirring for 30 min. After that, 4 ml TIP was added to the solution under vigorous stirring at −20 °C. When the clear solution turned into milky white suspension, the suspension was kept at room temperature without stirring to stand for 14 h. After centrifugation (8000 rpm, 5 min), the precipitate was dispersed in 200 ml ethanol, and then stirred for 2 h at 70 °C. After stirring and cooling to room temperature, as-prepared MTNs (the precipitation) were collected by centrifugation. To completely remove F127, MTNs were washed three times by repeating the same method, and then dried at 40 °C for 48 h to remove ethanol.

2.2.2. Synthesis of MTNs-NH_2

100 mg of MTNs were added to 20 mL of dry toluene, and then sonicated for 1 h to form a homogeneous suspension. After that, 5 mL APTES was added, and the solution was allowed to stir under vacuum at 80 °C for 24 h under N_2. After reaction for 24 h, MTNs-NH_2 was collected by centrifugation (8000 rpm, 5 min), purified by washing three times with ethanol and then dried at 40 °C for 24 h in vacuum.

2.2.3. Synthesis of MTNs-Maleimide (MTNs-Mal)

Maleimide group was linked to MTNs-NH_2 via GMBS [18]. In total, 50 mg MTNs-NH_2 was suspended in 4 ml ethanol and then sonicated for 20 min. Then 50 μl N-(4-Maleimidobutyryloxy) succinimide (GMBS, 22.5 mM) was added to the solution of MTNs-NH_2, and then stirred at room temperature for 24 h. After reaction for 24 h, MTNs-Mal was collected by centrifugation (10,000 rpm, 15 min), the precipitate was washed three times with ethanol, and then dried at 40 °C for 48 h to remove ethanol.

2.2.4. DOX loading

MTNs-Mal (30 mg) was added to DOX methanol solution (10 ml, 2 mg/ml) and sonicated at room temperature for 2 h and then sonicated using an ultrasonic cell disruption system (200 W, 10 times), and then the nanosuspension was moved to a rotary evaporator, and removed the methanol slowly at 40 °C. After that, the precipitation was washed by 20 ml water to remove the un-loaded DOX, and the product (MTNs-Mal/DOX) was obtained by centrifugation (10,000 rpm, 15 min). The amount of free DOX was determined by high performance liquid chromatography (HPLC, 1100 Agilent, USA) with the following conditions: an Eclipse XDB-C18 column (150 mm × 4.6 mm, 5.0 μm); mobile phase sodium acetate solution (0.02 mol/l)/acetonitrile 80:20; column temperature 40 °C; fluorescence detector with the excitation and emission wavelengths set at 475 nm and 560 nm, respectively; flow rate 1.0 ml/min; and injection volume 20 μl. mloaded DOX = 20 mg-mfree DOX. The DOX encapsulation efficiency (EE) was calculated using the following formula:

$$EE = \frac{W}{W0} \times 100\%$$

W was the weight of DOX in NBMTNs. W0 was the weight of DOX initially added in NBMTNs preparation.

2.2.5. DNA Capping

DOX loaded MTNs (30 mg) was dispersed in 10 ml water, and then the dsDNA (200 bp, 0.15 mg/ml, 1 ml) was added, after stirred at 4 °C for 24 h, the resulting product (NBMTNs) was obtained by centrifugation (10,000 rpm, 20 min).