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Self-crosslinkable and intracellularly decrosslinkable biodegradable micellar nanoparticles: A robust, simple and multifunctional nanoplatform for high-efficiency targeted cancer chemotherapy

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ABSTRACT

Nanomedicines based on biodegradable micelles offer a most promising treatment for malignant tumors. Their clinical effectiveness, however, remains to be improved. Here, we report that self-crosslinkable and intracellularly decrosslinkable micellar nanoparticles (SCID-Ms) self-assembled from novel amphiphilic biodegradable poly(ethylene glycol)-*b*-poly(dithiolane trimethylene carbonate) block copolymer achieve high-efficiency targeted cancer chemotherapy *in vivo*. Interestingly, doxorubicin (DOX)-loaded SCID-Ms showed favorable features of superb stability, minimal drug leakage, long circulation time, triggered drug release inside the tumor cells, and an unprecedented maximum-tolerated dose (MTD) of over 100 mg DOX equiv./kg in mice, which was at least 10 times higher than free drug. The *in vivo* studies in malignant B16 melanoma-bearing C57BL/6 mice revealed that DOX-SCID-Ms at a dosage of 30 mg DOX equiv./kg could effectively suppress tumor growth and prolong mice survival time without causing obvious systemic toxicity. Moreover, DOX-SCID-Ms could be readily decorated with a targeting ligand like cRGD peptide. The biodistribution studies showed that cRGD20/DOX-SCID-Ms had a high tumor accumulation of 6.13% ID/g at 6 h post injection, which was *ca.* 3-fold higher than that for clinically used pegylated liposomal doxorubicin (DOX-LPs). Accordingly, cRGD20/DOX-SCID-Ms exhibited significantly better therapeutic efficacy and lower side effects than DOX-LPs in B16 melanoma-bearing mice. These self-regulating biodegradable micellar nanoparticles offer a robust, multifunctional and viable nanoplatform for targeted cancer chemotherapy.

1. Introduction

Nanomedicines based on biodegradable micelles and nanoparticles offer a most promising treatment for malignant tumors [1–4]. Their clinical effectiveness, however, remains to be further improved by increasing their systemic stability, tumor accumulation and selectivity, and/or control over drug release [5–8]. In the past years, various stimuli-responsive multifunctional nanosystems have been developed to achieve enhanced therapeutic efficacy in different tumor models [9–13]. The typically sophisticated design and fabrication, potential safety and toxicity concerns, and high cost, however, preclude their clinical translation [14–17]. Lack of safe, simple and therapeutically effective nanovehicles has been a long challenge for the clinical translation of anticancer nanomedicines [18–22].

In this study, we have designed and developed self-crosslinkable and intracellularly decrosslinkable micellar nanoparticles (SCID-Ms) for high-efficiency targeted cancer chemotherapy *in vivo* (Scheme 1). SCID-Ms are self-assembled from novel amphiphilic biodegradable poly(ethylene glycol)-*b*-poly(dithiolane trimethylene carbonate) (PEG-*b*-PDTC) block copolymer, in which the hydrophobic PDTC block is composed of biodegradable poly(trimethylene carbonate) (PTMC) main chain and multiple dithiolane rings at the side. PTMC and its derivatives with excellent biocompatibility and biodegradability have been widely used for various biomedical applications such as absorbable sutures, tissue engineering, and drug and gene delivery [23–28]. The pendant dithiolane ring is analogous to that of the lipoic acid, a natural antioxidant produced by the human body and a drug used for Alzheimer's disease and diabetes [29]. Notably, dextran–lipoic acid and hyaluronic acid–lysine–lipoic acid conjugates furnished, in the presence of a catalytic amount of dithiothreitol, reduction-sensitive reversibly crosslinked nanoparticles [30–31]. The past years have witnessed a remarkable development of reduction-sensitive nanoparticles for triggered cytoplasmic drug and gene delivery [32–41], based on the fact that there exists 2–3 orders magnitude higher redox potential in the cytoplasm of tumor cells than in the blood circulation [42–43]. Intriguingly, these novel self-crosslinkable bioresponsive biodegradable micellar nanoparticles are not only multifunctional but also simple and easy to prepare, which makes them unique and particularly promising for translational research.

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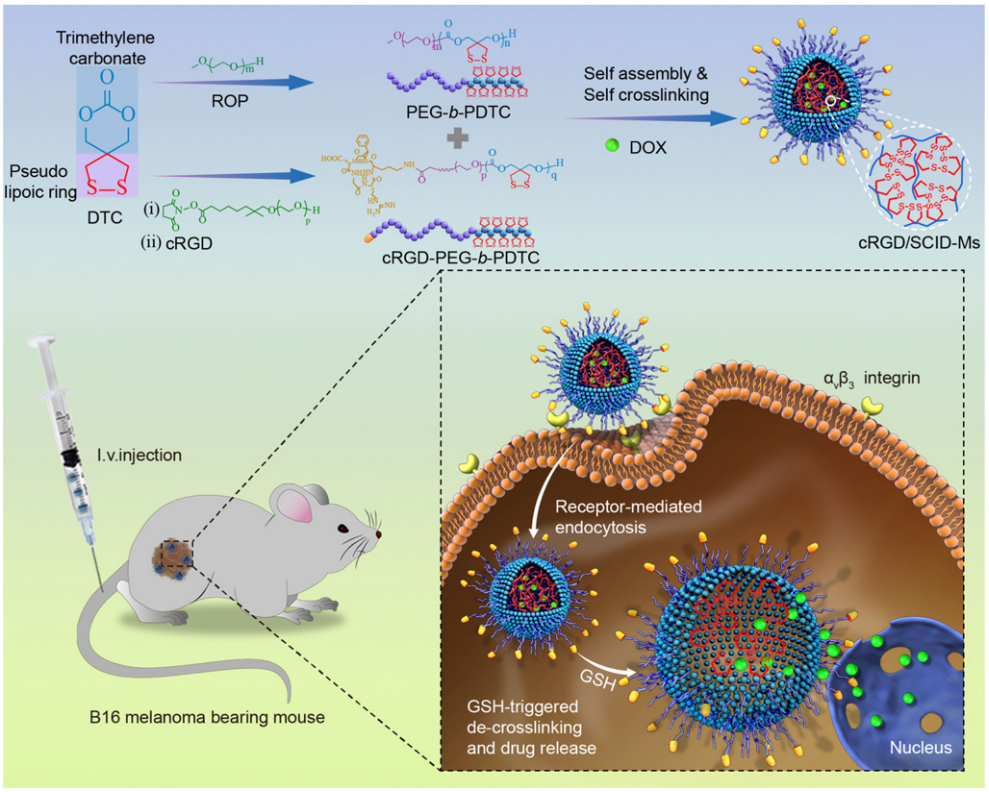


Scheme 1. Schematic diagram depicting formation and *in vivo* tumor-targeting doxorubicin delivery of cRGD-decorated self-crosslinkable and intracellularly decrosslinkable micellar nanoparticles (cRGD/SCID-Ms).

2. Materials and methods

2.1. Materials

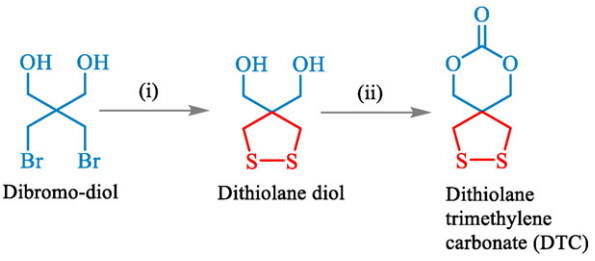
Methoxy poly(ethylene glycol) (MeO-PEG-OH, M_n = 5.0 kg/mol, PDI = 1.03, Fluka) and *N*-hydroxysuccinimide activated poly(ethylene glycol) (NHS-PEG-OH, M_n = 6.5 kg/mol, PDI = 1.04, Suzhou Nord Derivatives Pharm-tech Co. Ltd) were dried by azeotropic distillation from anhydrous toluene. Dichloromethane (DCM) was dried by refluxing over CaH_2 and distilled prior to use. Zinc bis [bis(trimethylsilyl) amide] (97%, Aldrich), C(RGDfK) (cRGD, 98%, China Peptides Co., Ltd.), glutathione (GSH, 99%, Roche), and doxorubicin hydrochloride (DOX·HCl, >99%, Beijing Zhongshuo Pharmaceutical Technology Development) were used as received.

2.2. Characterization

The polymer structures were characterized using ^{1}H NMR on a Unity Inova 400 spectrometer operating at 400 MHz. The chemical shifts were calibrated against residual solvent signal. The molecular weight and polydispersity of copolymers were determined by a Waters 1515 gel permeation chromatograph (GPC) instrument equipped with two linear PLgel columns (500 Å and Mixed-C) following a guard column and a differential refractive-index detector. The measurements were performed using DMF as an eluent at a flow rate of 1.0 mL/min at 30 °C and a series of narrow polystyrene standards for the calibration of the columns. The size and size distribution of micellar nanoparticles were determined at 25 °C using dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern Instruments) equipped with a 633 nm He–Ne laser using back-scattering detection. Zeta potential measurements were carried out using a Zetasizer Nano-ZS instrument (Malvern) equipped with a standard capillary electrophoresis cell. The measurements were performed in triplicate. Transmission electron microscopy (TEM) was performed using a Tecnai G220 TEM operated at an accelerating voltage of 120 kV. The samples were prepared by dropping 10 μL of 0.2 mg/mL micellar nanoparticle suspension on the copper grid followed by staining with phosphotungstic acid (1 wt.%). The CLSM images were taken on a confocal laser scanning microscope (TCS SP5). The absorbance of dithiolane rings in the micellar nanoparticles at 330 nm was monitored using a double-beam UV–visible system (UH5300 Hitachi). PEG-PCL micellar nanoparticle dispersions were used for calibration.

2.3. Synthesis of dithiolane-functionalized cyclic trimethylene carbonate (DTC)

DTC was synthesized in two steps (Scheme 2). Firstly, to a solution of 2,2-bis (bromomethyl)-1,3-propanediol (20 g, 0.08 mol) in DMF (350 mL) under stirring was added NaSH·H_2O (28.26 g, 0.38 mol). The reaction was allowed to proceed at 75 °C for 48 h under constant stirring. The solvent was removed by distillation under reduced pressure. The residue was diluted with D.I. water (300 mL) and extracted with EtOAc (3 × 250 mL). The organic phase was dried over anhydrous $MgSO_4$. The solvent was evaporated to yield 2-(1,2-dithiolan-4-yl)-1,3-



Scheme 2. Synthesis of dithiolane-functionalized cyclic carbonate (DTC). Conditions: (i) NaSH, DMF, 75 °C, 48 h, and in air for 24 h; (ii) ethyl chloroformate, Et_3N, THF, 0 °C, 4 h.