Supporting Information

Red blood cells membrane coated silica nanoparticles co-delivering DOX and ICG for effective lung cancer therapy

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Experimental Section

Materials

Methyl thiazolyl tetrazolium (MTT), tetraethyl orthosilicate (TEOS), doxorubicin (Dox), indocyanine green (ICG), Triton X-100, paraformaldehyde, Fluo-4 AM, N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPS) and propidium iodide (PI) were purchased by Sigma-Aldrich (St. Louis, MO, USA). Chemicals and regents otherwise stated were obtained from Aladdin Co., Ltd (Shanghai, China) and of analytical pure.

Establishment of cell and animal models

A549/Dox (human lung carcinoma with resistance to Dox) and NIH3T3 (mouse embryonic fibroblast) cell lines were provided by IBCB center of Shanghai (Shanghai, China). All cell lines were growed and operated by standard protocol as reported. [1] Male Balb/c nude mice at the age of 6-8 weeks were provided by Institute of Model Animal Wuhan University (Wuhan, China). The establishment of A549/Dox tumor xenograft model follows the protocol of previous report. [2] The adopted animal procedures in our experiments were all approved by the IEC (Institutional Ethics Committee) of Zunyi Medical College.

Multi-cellular tumor spheroid (MCTS) model

The MCTS was established following previous study. [3] In brief, autoclaved agarose solution was added into the 96-well plate (Corning, USA) to create the gel pads. Then A549/Dox and NIH3T3 cells (cell density: 2×10^3/well) were mixed at the ratio of 1:1 and seeded into the plate for the form of MCTS.

Preparation of RS/I-D

The synthesis of ICG and Dox loaded SLN (SLN/I-D) was conducted in a water-in-oil microemulsion using a previous reported protocol. [4] Firstly, a water-in-oil microemulsion (10 mL) containing Dox and ICG was prepared. Then TEOS (5 mg), AEAPS (2 mg) and NH_4OH (100 μL) were successively added into the microemulsion to initiate the reaction. After 24 h of reaction, SLN/I-D was precipitated by excess ethanol and collected by centrifugation (3000 rpm, 10 min, CR26, Hitachi, Japan).

The isolation of RBM from red blood cells (RBC) cells was performed according to previous report. [5] In brief, the RBCs were collected and concentrated using centrifugation. Afterwards, cells was homogenized in 1 mL of extracting buffer (PBS, 0.0001M) and further centrifuged (10000g, 10 min), followed by a second ultracentrifugation (100 000g, 60 min) to finally obtain the RBM. All procedures were performed at 4 °C. The protein concentration of RBM was quantified using a BCA kit (Beyotime, Shanghai, China) according to the manufacturer's instructions.

The RBM was then deposited onto the surface of SLN/I-D to construct RS/I-D. Briefly, 250 μL of SLN/I-D (1 mg/mL) was mixed with RBM solution under vortex (w/w ratio of 5). Afterwards, the mixture was subjected to probetype sonication (100 W, 5 min). The mixture was further centrifuged (10000g, 10 min) to collect RS/I-D.

Characterization

The size and surface potential of RS/I-D was performed by a Zeta-sizer analyzer

(ZS90, Malvern, UK).

The drug loading content (DLC) of RS/I-D was studied by emerging nanoparticles in mixed solution of HCl and ethanol for 48 h. [6] After being centrifuged (10000 rpm, 30 min), the Dox and ICG contents within the supernatant were assessed by UV spectrometer Agilent Cary 3500 (Agilent, California, USA) at 480 nm and 780 nm, respectively.

The release profile of Dox and ICG from RS/I-D, respectively, was assessed, as referred to previous report. [2] Briefly, RS/I-D were charged into in dialysis bag (MWCO: 7 KDa) and incubated with proper amount of PBS with (with or without laser irradiation 1W/cm^2 for 5 min, respectively) 0.1% Tween 80. The release experiment was conducted using a shaking bath (SY-2230; Crystal Technology, Texas, USA) at 37 °C. The drug content in the release medium was determined by UV at predetermined time intervals.

Stability and hemolysis assay were performed according to previous report. [7] In brief, for stability test, the freshly prepared RS/I-D solution was diluted with PBS at the volume ratio of 1:10. The change in particle size was recorded by Zetasizer Nano ZS90 at predetermined time intervals for up to 48 h. For hemolysis assay, RBCs were firstly obtained from Balb/c and diluted to 2% suspension with saline solution. Then the RBCs were formulated as a 2% suspension with saline solution. The RS/I-D was added into 2% RBCs suspension with same volume to achieve the designated concentrations (0.1, 0.25, 0.5, 0.75, 1 mg/mL) and incubated at 37 °C for 1 h. Besides, RBCs suspension was also incubated with saline and distilled water under the same condition as negative (0% hemolysis) and positive controls (100% hemolysis), respectively. After that, all the samples were centrifuged at 3000 rpm for 10 min and the absorption values (represented the counts of released hemoglobin) of same volume of supernatants were measured at 545 nm by UV spectrophotometer.

Cytotoxicity assay and cell cycle

The cytotoxicity of blank carrier (10-200 μg/mL) and RS/I-D (Dox: 0.25-5 μg/mL, the weight ratio between Dox and ICG was fixed at 1) on A549/Dox cells (48 h) was performed by classic MTT assay. The blank cells without any treatment were selected as control.

MCTS with diameters of 300-400 μm were treated with fresh medium containing different formulation (Dox: 3.5 μg/mL) for 5 days. The diameter changes of MCTS was recorded by the optical microscope EVOS XL Core (Thermo-Fisher, Massachusetts, USA).

Cell Cycle Kit (Beyotime, Shanghai) was used to mark the cell cycle of cells as manufacturer's instructions. Afterwards, cell cycle changes were determined by flow cytometry (FCM, ACEA NovoCyte, Agilent, California, USA).

Cellular mechanisms

A549/Dox cells were seeded into 6-well plates with 70% confluence and then cultured with free Dox, free ICG, RS/D, or RS/I. For competitive binding assay, cells were treated with excessive RBM for 2 h prior to the addition of various formulations.

Afterwards, cells were subjected to FCM analyzation at prearranged time intervals as reported previously [8] and observed under confocal laser scanning microscope (FV3000, Olympus, Tokyo, Japan).

Western blot

RIPA lysis buffer (Thermo Fisher, USA) was used to extract total protein of samples, then the protein content was quantified by the BCA kit. Afterwards, samples were transferred onto PVDF membrane, cultured with corresponding first antibodies (Abcam, UK), then incubated with IRDyeR680CW-labeled second antibody (Abcam). The visualization of blots was performed by densitometer (E-Gel Imager, Thermo-Fisher, USA) and the experiment were performed for 3 times in parallel.

In vivo antitumor study

In vivo antitumor study of RS/I-D was assessed using A549/Dox tumor-bearing mice. The mice were randomly divided into 4 groups (n = 6): 1) saline (as control); 2) RS/D; 3) RS/I; 4) RS/I-D. Protocols were adopted from previous report. [9] Briefly, mice were intravenously administered with various formulations every other day for 7 times, each formulation contained the same dose of drugs (Dox and ICG: 5 mg/kg). The irradiation was performed 24 h post injection (1 W/cm^2 for 5 min at the tumor tissue). At the end of the test, the mice were sacrificed and the tumor tissues from each group were embedded in paraffin, sliced at 10 μm of thickness and then subjected to TUNEL staining using the TUNEL kit (Roche) as instructed. The images were taken for one tumor at three different regions.

Refernce

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