Supporting Information

Long circulation and tumor-targeting biomimetic nanoparticles for efficient chemo/photothermal synergistic therapy

Yifan Zhang[a], Xuanyu Yue[a], Shengchao Yang[*a], Xianglong Li[b], Lin Cui [a], Xiaobin Cui[a], Yue Shi[c], Zhiyong Liu[*a], Xuhong Guo [a, d], Yongsheng Li [*a, b]

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1.Experimental Section

1.1 Materials

Ethyl orthosilicate (TEOS), cyclohexane, cetyltrimethylammonium bromide (CTAB), Tris-Hydrochloride Buffer, Dopamine hydrochloride, deionized water, carbamide, ethanol, BALB/c mice blood, 1,2-distearoyl-sn-glycero-3-phosphoethanol amine-N-[biotinyl(polyethylene glycol)-2000](DSPE-PEG_{2000}-Bio), Bovine Serum Albumin (BSA), fetal bovine serum, doxorubicin hydrochloride (DOX), triethylamine, HeLa cell, RAW264.7 cell, DMEM cell culture medium, Hoechst 33342, Cell Counting Kit-8(CCK-8), dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS), penicillin, trypsin, 3,3'-Dioctadecyloxacarbocyanine perchlorate (DiO), glycocoll, ammonium persulfate, Tris, acrylamide, N, N'-methylene bisacrylamide, BCA protein assay kit, Hematoxylin and Eosin Staining Kit. All other reagents were of analytical purity

1.2 Characterization

Transmission electron microscopy (TEM) images were taken on an HT7700 microscope (Hitachi, Japan) and scanning electron microscope (SEM) images were detected by Hitachi SU8010. PL spectra were detected on Flex One. For the micromeritics A500 device, the nitrogen adsorption isotherm was run at 77 K. UV-vis spectrometry was recorded with the UV755B. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protein strips were observed through the gel imaging system Gel Doc XR+. Microplate reader K3plus was used to detect protein content, cytotoxicity and hemolysis. Liposome extruders were used to coat cell membranes(Avestin, LF-1, Canada). The diameter and Zeta potential were performed by Malvern Zetasizer Nano-ZS instrument. Confocal Laser Scanning Microscopy (CLSM) images were taken by Zeiss CLSM510. Flow Cytometry (BD FACSCalibur) were purchased from the USA. Infrared light thermography were taken on by FLIR T840. Fiber-coupled semiconductor lasers emited a beam at 808 nm.

1.3 Fabrication of mesoporous silica nanoparticles (MSN)

Mesoporous silica was synthesized by soft template method. 3.1g TEOS was added into 30 ml of cyclohexane and stirred evenly. 2.7g CTAB and 6 g carbamide were dissolved in 30 ml of deionized water, stirred and transferred to PTFE counter. It should be washed three times with deionized water and dried in air at 70 °C for 12 hours. Then the mesoporous silica white powder (MSN) was obtained by sintering in a muffle furnace at 550 °C for 3 h to remove the surfactant template.

1.4 Mesoporous silica coated with polydopamine membrane (PDA@MSN)

0.04 g of MSN powder and Tris○HCl (pH=8.5) were mixed to give a uniform distribution of MSN. Add 0.02 g of dopamine solid in a glass reaction flask and 4 mL

of MSN-Tris○3HCl (pH=8.5) dispersion to the flask. The reaction was carried out with rapid stirring, protected from light for 48 hours, washed three times by centrifugation and the supernatant was clear and transparent.

1.5 PDA@MSN loaded anticancer drugs DOX(PDA@MSN-DOX)

The 10 mg sample and different weights of DOX were added to 20 mL water and all sample were stirred for 12 h to load the anticancer drug by electrostatic action. The concentrations of DOX are respectively 200 μg/mL, 250 μg/mL, 300 μg/mL, 350 μg/mL, 400 μg/mL. The adsorption capacity of the loaded DOX on nanoparticles was further quantified by the determination of the supernatant absorbance. The drug loading content (DLC) was calculated.

1.6 Preparation of Targeting Substance-Inserted Erythrocyte Membrane (Bio-RBCm).

Erythrocyte membrane vesicles were prepared by published articles[16]. Firstly, 10 mL whole blood of BALB/c mice was collected and centrifuged at 2500 rpm for 10 min, and then 1○PBS was washed three times. Secondly, after adding 0.25○PBS to the centrifuged erythrocytes for 30 min, the pink pellet (RBC ghosts) was obtained after centrifugation at 10000 rpm. The collected RBC ghosts were extruded through 800nm, 400 nm and 200 nm polycarbonate (PC) porous membranes, the RBC membrane-vesicles (RBCm) were successfully harvested. The resulting erythrocyte membrane vesicle structure and 20 μg DSPE-PEG_{2000}-Bio were mixed and fully stirred 2 h at 4 ^{o}C. The byproduct was removed by centrifugation at 12000 rpm for 10 min. The biotin modified erythrocyte membrane were ultrasounded for 3 min (40 kHz, 500 W), and extruded through 800 nm, 400 nm, 200 nm PC membranes using the Avestin extruder to get RBCm[5, 19].

1.7 Preparation of Bio-RBCm@PDA@MSN-DOX

The product of Bio-RBCm vesicles were mixed with the dispersed PDA@MSN -DOX, extruded through 800 nm, 400 nm, 200 nm PC membranes using the Avestin extruder to get Bio-RBCm@PDA@MSN-DOX. BCA Protein Assay Kit was introduced to verify RBC coating on the surface of MSN-DOX and measured at 540 nm. Besides, a cell membrane dye, DiO, was added to label Bio-RBC@PDA@MSN-DOX. Due to the specific binding between fluorescent probe and membrane-bounded protein, a green light was emitted and could be detected by confocal laser scanning microscopy. Cell membrane proteins were detected by gel imaging system.

1.8 *In Vitro* DOX Release Investigation

Dialysis tubes (MWCO: 1000 Da, Millipore) with 2 mL of PDA@MSN-DOX and Bio-RBCm@PDA@MSN-DOX were directly put into 25 mL PBS (pH=7.4) and PBS (pH=5.0) at 37 ℃ for 150 h at 200 rpm, while adding near infrared illumination(808 nm) as a variable. The medium was withdrawn and replaced with an equal amount of

fresh medium at the appointed time. The absorbance of the solution was detected by UV-*vis* spectrometry. The cumulative drug release rate is calculated as follows:

$$Er(\%) = \frac{V_e \sum_1^{n-1} c_i + V_0 c_n}{m_{DOX}} \times 100 \quad (2\text{-}1)$$

1.9 Protein adsorption studies

The protein amount of Bio-RBCm@PDA@MSN-DOX is also evaluated by BCA assay kit. Briefly, the test reagent contain 25 mM BCA, 3.2 mM $CuSO_4$. Samples was incubated at 70°C for 30 minutes. At the end of the colour development process, the substance to be tested is left 25°C. The absorbance reading at 562nm was measured using an Microplate reader. The BSA was used as a standard.

1.10 Hemolysis assay

Mouse whole blood was centrifuged at 3000 rpm for 5 min and washed five times with PBS solution to get pure erythrocytes. Afterward, 0.25 mL of 6% erythrocytes (BALB/c mice) was mixed with 0.25 mL of water, RBCm vesicle, PDA@MSN-DOX, RBC@PDA@MSN-DOX, Bio-RBC@PDA@MSN-DOX solution and incubated at 37 °C for 4 h. Red blood cells were mixed with ultrapure water, which was defined as a positive control. The samples were centrifuged and the supernatant was aspirated. An Microplate reader was used to determine the absorbance of the supernatant. The percentage of hemolysis was calculated following equation.

$$\text{Hemolysis } (\%) = (I/I_0) \times 100 \quad (2\text{-}2)$$

where I represents the absorbance of supernatant for nanoparticles, and I_0 is the absorbance of erythrocytes after complete hemolysis in pure water.

1.11 Exploration of photothermal performance

The effects on photothermal properties were investigated with different concentration, different power density and nanoparticle stability.

$$\eta = \frac{\mathrm{hA}\Delta \mathrm{T_{max}} - \mathrm{Q_s}}{I(1 - 10^{-A_\lambda})} \quad (2\text{-}3)$$

$$\theta = \frac{\Delta T}{\Delta T_{\max}} \quad (2\text{-}4)$$

where h is the heat transfer coefcient, A is the surface area of the container, T_{max} is the equilibrium temperature, I is the incident laser power (2 W cm^{-2}), and A_λ is the absorbance of 200 μg mL^{-1} Bio-RBCm@PDA@MSN-DOX at 808 nm. Qs is the heat associated with the light absorbance of the solvent. The value of hA is derived from Eq. 2-3.

1.12 Cell Culture

HeLa cells and RAW264.7 cells were cultured in DMEM medium containing 10% FBS and 1% streptomycin/penicillin, respectively. Cells were cultured at 37 ºC in a humidified atmosphere with 5% CO_2.

1.13 The biocompatibility of Bio-RBC@PDA@MSN and the *vitro* Cytotoxicity of Bio-RBC@PDA@MSN-DOX

We put HeLa cells (5×10^3 cells) into each well of a 96-well plate and incubated for 12h. Then the medium was discarded and different concentrations of Bio-RBCm @PDA@MSN-DOX were added. After 6h incubation, the culture solution was removed. After adding new medium and continuing the incubation for 24h, the cells were mixed with a solution containing 10 μL CCK-8. After 1h incubation, the absorbance of the solution was measured using the Microplate reader.

1.14 *In Vitro* Immune Escape and targeting ability

We put HeLa and RAW264.7 cells (1×10^5 cells per well) into each well of a 12-well plate and incubated for 6h. Then the medium was discarded. Bio-RBCm@PDA@ MSN-DOX NPs were added and incubated at 37 °C for 12 h. Then, the medium was removed and cells were washed three times. Then cells were stained with fluorescent dyes and the amount of nanoparticles entering the RAW264.7 and HeLa were observed by Confocal Laser Scanning Microscopy.

1.15 *In Vivo* Infrared Thermal Imaging of Bio-RBCm@PDA @MSN-DOX

The BALB/c mice were obtained from SLAC (Shanghai, China). The animal experiments were strictly carried out following the “National Animal Management Regulations of China” and approved by the Hospital of Fujian Medical University. Tumor-bearing mice were established by injection of Hela cells at a density of 1×10^6 per mouse. With time, mice developed distinctive masses of tumor under the skin. When the tumor size reached 100 mm^3, Bio-RBCm@PDA@MSN-DOX(80 μL, 4 mg/mL) was injected into the experiment mice through the tail vein, and the *in vivo* Infrared Thermal imaging was monitored with the 808 nm NIR light after 24 h injection.

1.16 Internal long-circulation performance and targeted performance test

Male BALB/c mice (5 weeks old) were injected intravenously with PDA@MSN-DOX, RBCm@PDA@MSN-DOX or Bio-RBCm@PDA@MSN-DOX nanoparticles (50 mg kg^{-1}). Venous blood was collected at the indicated time points. Twenty-four hours after injection, mice were subjected to neck-breaking and execution, and major organs and tumors were collected and lysed in hydrofluoric acid. Silica content in blood and organs was determined using ICP-MS. The amount of nanoparticles was

calculated using a standard curve based on the increase in elemental silicon content in the blood or organs.

1.17 *In Vivo* Antitumor Effect

Male BALB/c mice (5 weeks old), logarithmic growth phase tumor cells HeLa cells were taken for subcutaneous injection into the back of the mice. On day 6 after injection, the tumor volume reached approximately 100 mm^3 (tumor volume = 1/2 × length × $width^2$). Mice were randomly divided into 8 groups (4 mice per group): (1) 1PBS solution as control; (2) 808 nm laser irradiation; (3) PDA@MSN-DOX+ light; (4) RBCm@PDA@MSN-DOX+ light; (5) Bio-RBCm@PDA@MSN+ light; (6) Bio RBCm@PDA@MSN-DOX; (7) Bio-RBCm@PDA@MSN-DOX+light; (8) DOX. 200 μL 1×PBS solution containing DOX (5 mg/kg), PDA@MSN-DOX, RBCm@PDA @MSN-DOX, Bio-RBCm@PDA@MSN-DOX nanoparticles were all administered to mice via tail vein at a concentration of 5 mg kg^{-1}. 24 h later, mice were irradiated with an 808 nm laser (2 W/ cm^2) for 3 min and local temperature changes during irradiation were monitored using a thermal infrared imaging camera. Tumor volumes and body weights were measured every other day. 15 days later, mice were executed and major organs and tumors were removed for H&E staining. Relative tumor volume (RTV) and tumor inhibition rate (TGI) were calculated according to the following equations:

$$\mathrm{RTV}(\%)=V_T / V_0 \tag{2-5}$$

$$\mathrm{TGI}(\%)=\frac{V_0 - V_T}{V_0}\times 100 \tag{2-6}$$

where V_0 is the control tumor volume and V_T is the post-treatment tumor volume.

2. Supplementary Figures

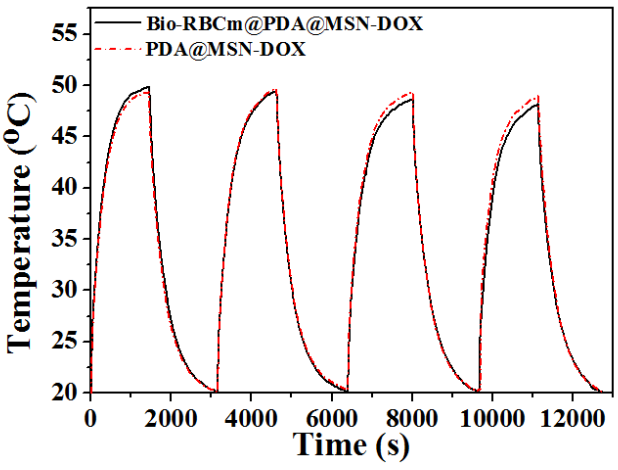

Figure S1:Photothermal stability of Bio-RBCm@PDA@MSN-DOX, upon four ‘‘On-to-Off’’ laser cycles with laser irradiation(2 W cm^{-2}) and the concentration of 200 μg/mL.

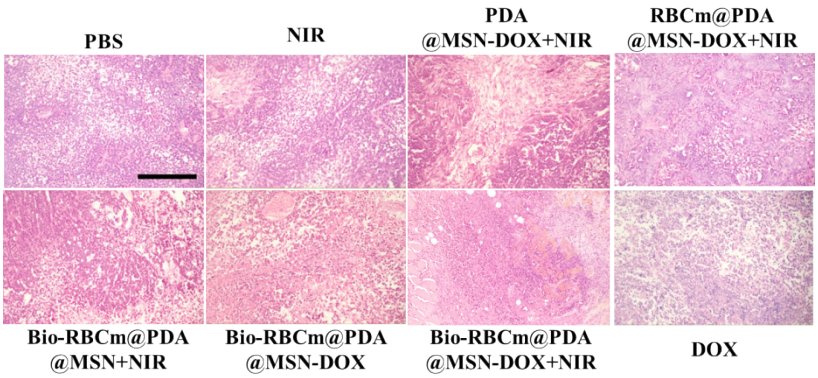

Figure S2. H&E staining of the tumor tissues slices for different treatment groups (scale bar = 50 μm).

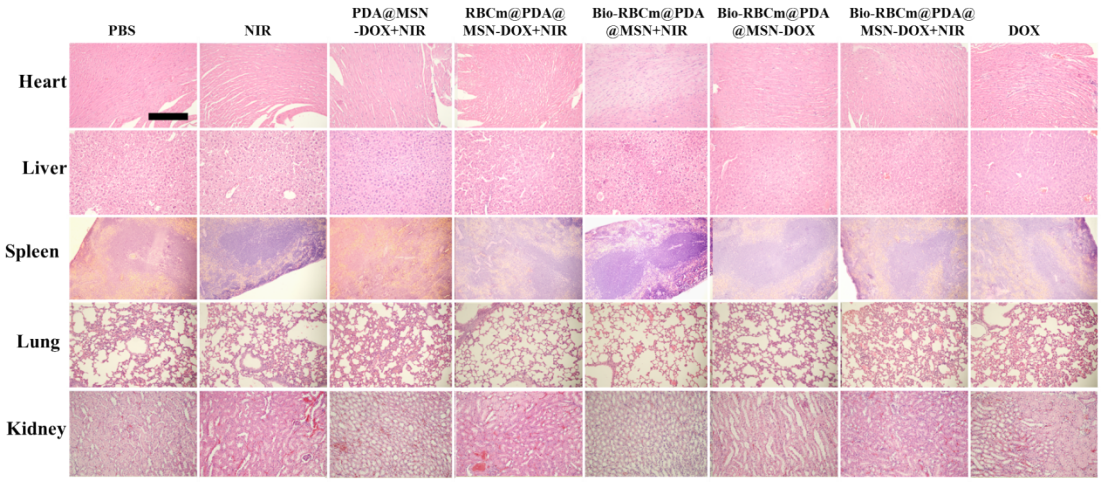

Figure S3. H&E staining of the major organs tissue slices for different treatment groups (scale bar = 50 μm).