Supporting information

For

Enhanced Blood Suspensibility and Laser-Activated Tumor-specific Drug Release of Theranostic Mesoporous Silica Nanoparticles by Functionalizing with Erythrocyte Membranes

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Supplementary Materials & Methods

Cell culture

Mouse macrophage RAW264.7 cells were purchased from Cell Bank of Shanghai, Chinese Academy of Sciences (Shanghai, China). RAW264.7 cells were grown in DMEM supplemented with 10% FBS, 100 U/mL penicillin G sodium and 100 mg/mL streptomycin sulfate. RAW264.7 cells were maintained at 37℃ in a humidified and 5% CO_2 incubator.

Characterization of RMSNs-Dox/Ce6

The ultraviolet-visible (UV-vis) absorption spectra of Dox and Ce6 in different formulations (Dox concentration of 50 μg/mL, Ce6 concentration 25 μg/mL) were measured on a UV-2450 spectrophotometer (Shimadzu, Japan). To investigate the effect of the laser power on the photoactivity of Ce6 in RMSNs-Dox/Ce6, RMSNs-Dox/Ce6 solution (Ce6 concentration of 10 μg/mL) was added with SOSG according to the manufacture's protocol, and then irradiated with a 655 nm laser with different power outputs. At appropriate points, the ROS generation was determined by measuring the fluorescence of SOSG (excitation = 525 nm).

To characterize the RBC membrane proteins on RMSNs surfaces, emptied RBCs, RVs and RVPNs were treated with SDS lysis buffer containing 1 mM PMSF. The protein concentration of lysates was determined using a BCA protein assay kit. MSNs were purified by centrifugation at 10000×g at 4℃ for 30 min to remove the free RVs. A 10 μg protein aliquot of each extract was separated by electrophoresis in a 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). For protein characterization, the polyacrylamide gel was stained by Coomassie brilliant blue according to the provided protocol before imaging. For western blot analysis, protein was transferred to a nitrocellulose (NC) membrane (Invitrogen, USA), blocked in 5% BSA TBS solution and blotted with CD47 extracellular domain primary antibody (R&D Systems, Inc., USA) and horseradish peroxidase (HRP)-labeledanti-rabbit IgG secondary antibody (Beyotime Co., Ltd., USA). ChemiDocTM MP imaging system (Bio-Rad, USA) was used for the detection of proteins.

Macrophage phagocytosis experiment

To investigate the cellular uptake of RMSNs in macrophage RAW 264.7 cells, Nile red was used to label MSNs. RAW264.7 cells were seeded in 24-well plates at 8×10^4 cells/well and allowed to attach for 24 h. Then 4T1 cells were incubated with different

nanoparticles at a Nile red concentration of 0.2 μg/well for 40 min, respectively. Then Hoechst 33342 (2.5 μg/mL) was added into each well for cell nucleus visualization. After 20 min, the extracellular fluorescence was quenched with 0.4% trypan blue for 2 min and washed with PBS. Cells were visualized under a confocal laser scanning microscopy (CLSM, Olympus, Japan), and the fluorescence analysis was acquired by a FACSCalibur system (Becton Dickinson, USA). To fabricate the Nile red loaded lipid-membrane-coated MSNs (LMSNs), the lipid membranes were fabricated by filming-rehydration method, phosphatidylcholine and cholesterol (9:1 mass ratio) were dissolved in acetonitrile, evaporated by rotary evaporation at 45℃ for 1 h and hydrated by distilled water. Then the Nile red-loaded MSNs suspensions were added, followed by probe sonication for 30 min at a power output of 100 W (JYD-650L, Zhixin Inc., Ningbo, China). Extra lipid films were removed by centrifugation at 10000g×20 min. Then resulting LMSNs were washed with distilled water for three times before use.

***In vivo* pharmacokinetics**

SD rats were randomly separated into three groups (n=3), and injected with free Dox, MSNs-Dox/Ce6 and RMSNs-Dox/Ce6, respectively, at the Dox dose of 5 mg/kg. 0.3 mL whole blood was collected in heparinized tubes at different time points (0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h), and then centrifuged to collect plasma. The content of Dox in plasma was measured by a microplate reader (Infinite F200, TECAN, Austria). The data were fit to a non-compartment model to analyze the pharmacokinetic parameters.

Hemolysis assays

Rabbit blood stabilized with EDTA was provided by Shanghai Experimental Animal Center (Shanghai). The whole blood was then centrifuged to remove plasma and buffy coat, and the red blood cells were then washed with PBS for three times. The resulting cells were diluted to 1/10 of their volume with sterile PBS solution. The diluted RBC suspension (300 mm^3) was then added to a) 1200 mm^3 of PBS as a negative control; b) 1200 mm^3 of deionized water as a positive control; c) 1200 mm^3 of MSNs-Dox/Ce6 or RMSNs-Dox/Ce6 suspensions at concentrations ranging from 15.63- 250 μg/mL. The mixtures were then gently vortexed and let to rest for 2 h at room temperature. The samples were then centrifuged (5000 r/min, 2 min), and the absorbance of the resulting supernatants at 541 nm were measured in a UV-2450 spectrophotometer

(Shimadzu, Japan). The hemolysis percentage of MSNs-Dox/Ce6 or RMSNs-Dox/Ce6 was calculated by the following equation: Hemolysis%= $(A_{sample}-A_{(-)contro})/(A_{(+)control}-A_{(-)control})$. (A = the absorbance intensity of UV-Vis spectra)

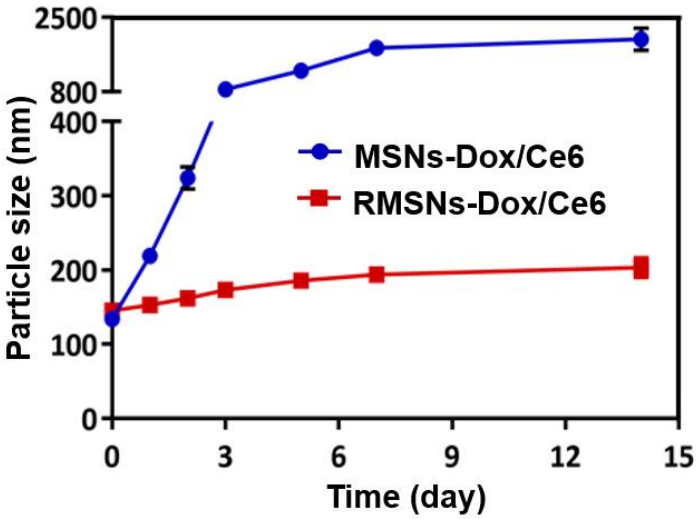

Figure S1. The particle size change of MSNs-Dox/Ce6 and RMSNs-Dox/Ce6 in 1640 medium containing 10% FBS. Data were presented as mean ± SD (n = 3).

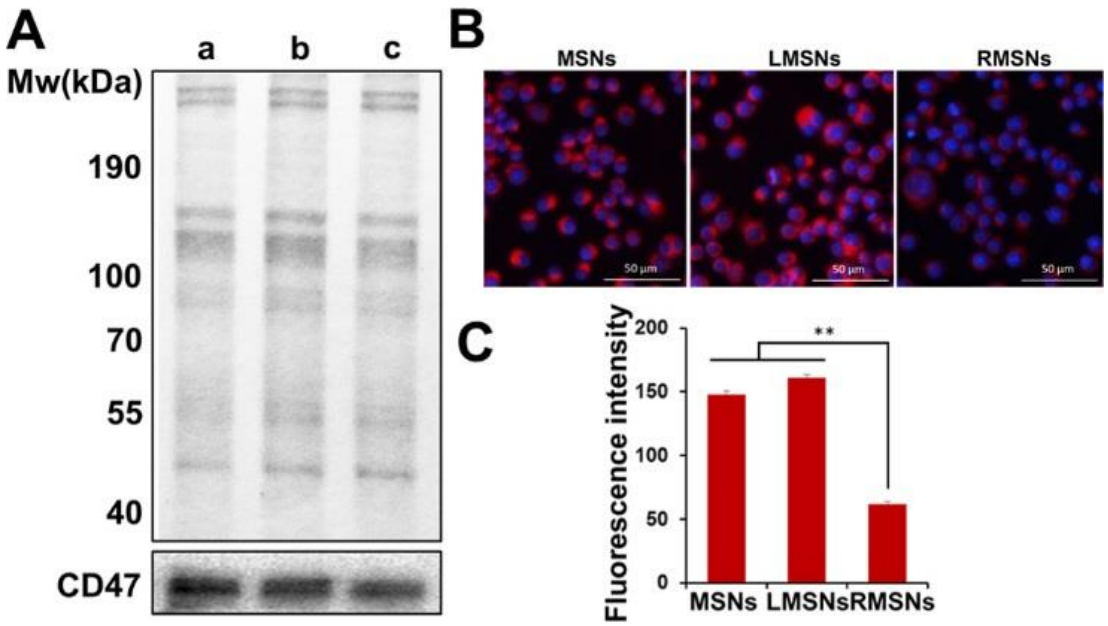

Figure S2. The RBC membrane protein retention and the reduced cellular uptake by macrophage cells of RMSNs. (A) RBC membranes protein retention of RMSNs. SDS-PAGE protein analysis (top) and western-blot analysis of CD47 protein (bottom) in a) emptied RBCs, b) RV, and c) RMSNs. (B) Representative fluorescence images of macrophage RAW264.7 cells treated with Nile red-loaded MSNs, LMSNs and RMSNs for 1 h. (nucleus: blue; Nile red: red) (B) Quantitative analysis of fluorescence intensity in RAW 264.7 cells. Data were presented as mean ± SD (n = 3), $^{**}P < 0.01$.

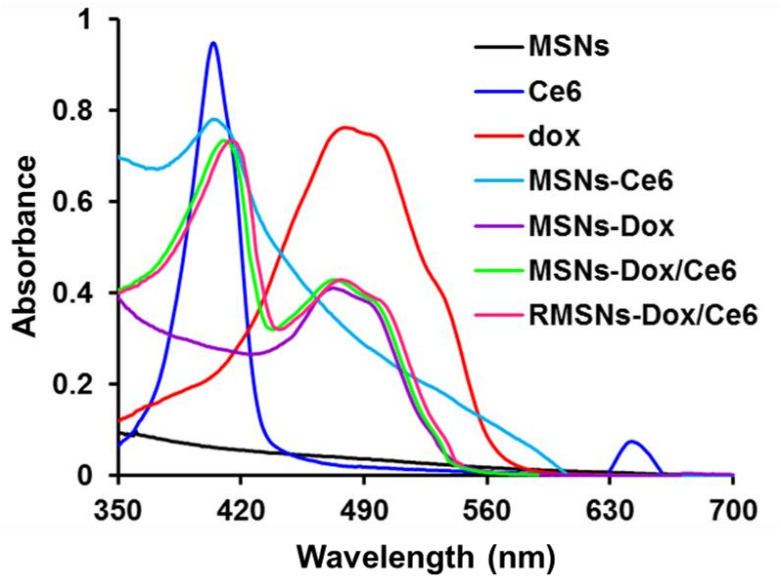

Figure S3. UV-vis spectroscopic measurement.

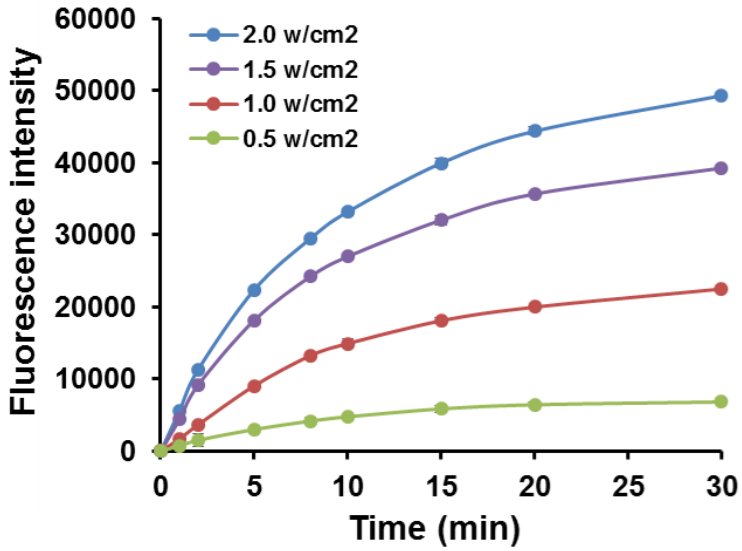

Figure S4. The increase of SOSG FL as a result of ROS generation of RMSNs-Dox/Ce6 under 655 nm laser irradiation at different laser powers.

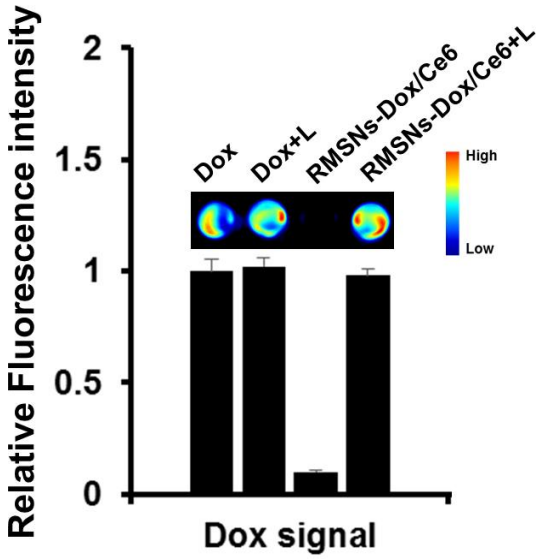

Figure S5. The laser-triggered Dox release of RMSNs-Dox/Ce6. The fluorescence images, and the corresponding fluorescence analysis of different Dox formulations, respectively. Data were presented as mean ± SD (n = 3).

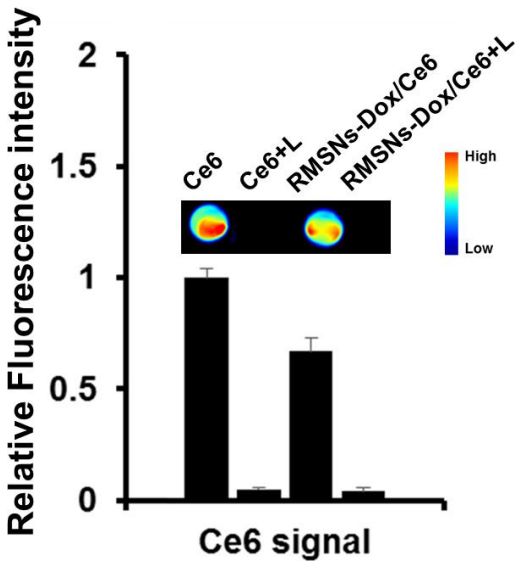

Figure S6. The influence of laser irradiation on the fluorescence signal of Ce6 in RMSNs-Dox/Ce6. The fluorescence images, and the corresponding fluorescence analysis of different Ce6 formulations, respectively. Data were presented as mean ± SD (n = 3).

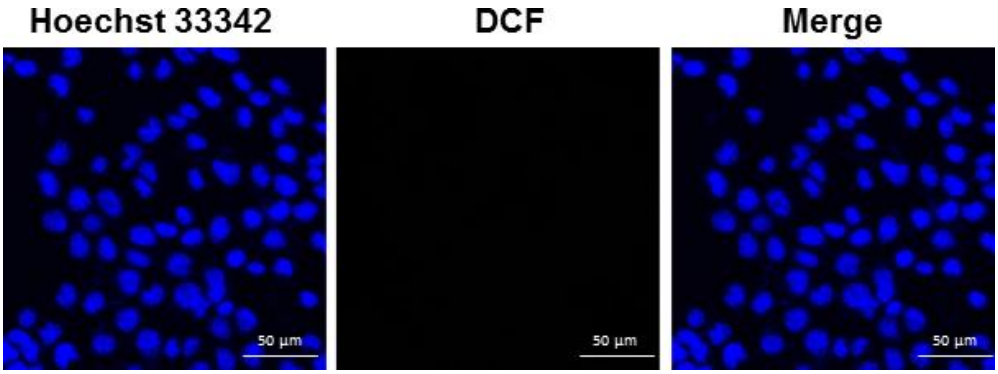

Figure S7. Representative fluorescence images of 4T1 cells treated with laser irradiated alone at a laser power of 2 W cm^{-2} for 5 min. Scale bar = 50 μm.

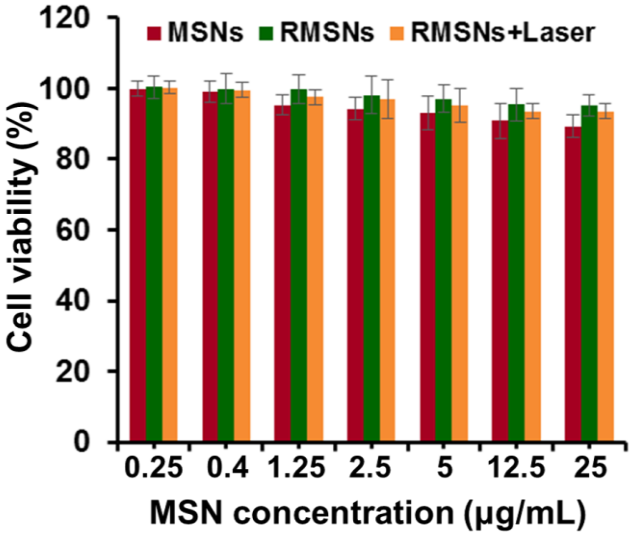

Figure S8. In vitro cytotoxicity of blank MSNs, RMSNs, and RMSNs+Laser at different concentrations, respectively. Data were presented as mean ± SD (n = 6)

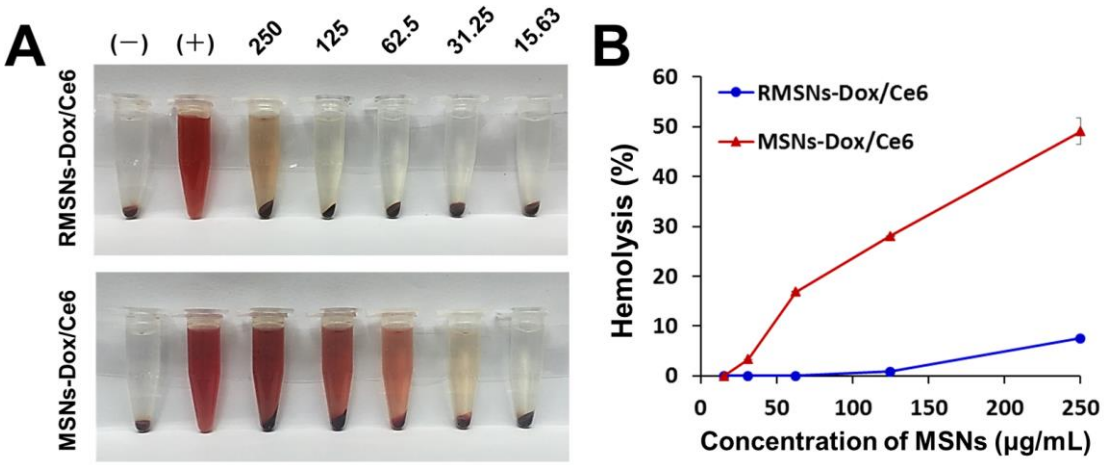

Figure S9. The hemolysis assays of MSNs-based formulations. (A). Digital photos illustrating the hemolysis effects of RBCs after 2 h incubation with RMSNs-Dox.Ce6 and MSNs-Dox/Ce6 at different concentrations. PBS and water were set as negative (−) and positive (+) controls, respectively. (B). The analysis of the hemolytic result. Data were presented as mean ± SD (n = 3).

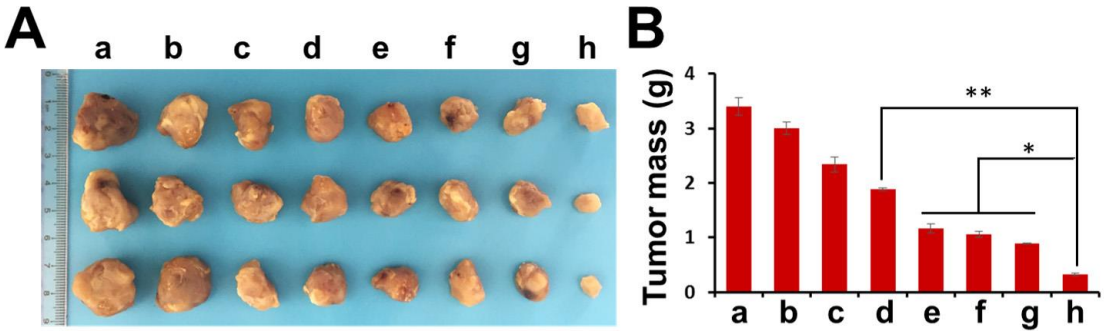

Figure S10. The representative digital photos (A) and the corresponding analysis (B) of the tumors excised from the tumor-bearing mice after 22 d treatment with (a) saline; (b) RMSNs+Laser; (c) Dox; (d) Dox/Ce6+Laser; (e) MSNs-Dox/Ce6+Laser; (f) RMSNs-Ce6+Laser; (g) RMSNs-Dox; (h) RMSNs-Dox/Ce6+Laser, respectively. Data were presented as mean ± SD (n = 6), $^{*}P < 0.05$, $^{**}P < 0.01$.

Table S1. Pharmacokinetic parameters of Dox after intravenous administration of Dox, MSNs-Dox/Ce6 and RMSNs-Dox/Ce6 to rats at the dose of 5 mg Dox/kg.

Parameter	Dox	MSNs-Dox/Ce6	RMSNs-Dox/Ce6
C_{max} [mg/L]	2.20 ± 0.39	5.10 ± 0.89	13.48 ± 2.35
$T_{1/2}$ [h]	0.27 ± 0.04	1.92 ± 0.56	18.17 ± 1.98
$MRT_{(0-\infty)}$ [h]	0.26 ± 0.16	2.43 ± 1.14	25.55 ± 2.68
$AUC_{(0-\infty)}$ [mg·h/L]	1.47 ± 0.76	10.29 ± 2.43	211.73 ±15.14
CL [L/[h/kg]]	3.41 ± 1.08	0.49 ± 0.08	0.02 ± 0.01