Electronic Supplementary Information

Targeted delivery of CuS nanoparticles through ultrasound image-guided microbubble destruction for efficient photothermal therapy

Zhengbao Zha, a Jinrui Wang, c Shuhai Zhang, a Enze Qu, c Hengte Ke a and Zhifei Dai b

a School of Life Science and Technology, Harbin Institute of Technology, Harbin 150080, China.
b Department of Biomedical Engineering, College of Engineering, Peking University, Beijing 100871, China. E-mail: zhifei.dai@pku.edu.cn
c Department of Ultrasonography, Peking University Third Hospital, Beijing 100083, China.

* Corresponding Author.

Experimental Details:

Chemicals:

Sorbitan monostearate (Span 60, Mw = 431), polyoxyethylene sorbitan monooleate (Tween 80, Mw = 1307) and poly (allylamine hydrochloride) (PAH, Mw ~56 000) were obtained from Sigma-Aldrich and used without further purification. Perfluoropropane (PFP) gas was purchased from Arkonic Gases and Chemicals (South Houston, TX, USA). Deionized water, with a resistivity of 18.2 MΩ·cm, was obtained from Milli-Q Gradient System (Millipore, Bedford, MA, USA) and used for all the experiments. The phosphate buffer saline (PBS) was prepared by mixing 8.010 g NaCl, 0.194 g KCl, 2.290 g Na2HPO4·12H2O and 0.191 g KH2PO4 in 1.0 L water and finally the pH value was adjusted to 7.4.

Preparation of ST68 MBs:

ST68 MBs were prepared as described by Basude et al [Ultrasound Med. Biol., 2000, 26(4): 621.]. Briefly, 1.48 g Span 60, 1.0 mL Tween 80 and 1.50 g NaCl were well mixed and suspended in 50 mL PBS, then probe-sonicated continuously by a 1.27 cm (1/2 inch) diameter titanium alloy horn (Sonicator 4000, Misonix, Farmingdale, NY, USA) with almost the maximum output amplitude setting under the atmosphere of PFP gas for 3 min. The resulting suspension was allowed to stand for
about 60 min to be separated into three layers. MBs were then collected from the middle layer and washed three times by PBS. Eventually, ST68 MBs were 1:1 (v:v) suspended in PBS, protected by PFP gas, sealed and stored at 4 °C.

**Synthesis of CuS NPs:**

The general procedure for the synthesis of CuS NPs in water was as described by Zhou et al [J. Am. Chem. Soc. 2010, 132(43): 15351.] with little modifications. Briefly, into 100 mL of an aqueous solution containing CuCl₂·2H₂O (0.0855 g) and sodium citrate (0.2279 g) was added 1 mL of sodium sulfide solution (Na₂S·9H₂O, 0.12009 g) with stirring at room temperature. The pale-blue CuCl₂ solution turned dark-brown immediately upon the addition of sodium sulfide. After 5 min, the reaction mixture was heated to 90 °C and stirred for 30 min until a dark-green solution was obtained. The mixture was transferred to ice-cold water. The citrate-coated CuS NPs (Cit-CuS NPs) were obtained and stored at 4 °C.

**Modification of ST68 MBs with CuS NPs:**

20 mL PAH solution (with a concentration of 1.0 mg mL⁻¹ containing 0.5 mol L⁻¹ NaCl) was added to the self-made centrifuge tube (with a drainage port at the bottom), which contained 5 mL ST 68 MBs suspension. The mixture was slightly shaken for 5 min to allow the sufficient adsorption reaction, and then stood at 4 °C for 30 min to let the MBs float to the top to form a foam-cake-like layer, under which the excessive polyelectrolytes were discarded. The MBs were then resuspended and washed by 20 mL PBS for twice by the same method. Thereafter, 20 mL 5mM Cit-CuS NPs solution was added and the excess CuS NPs were removed using the similar mix-float-wash steps. The adsorption procedure was repeated until the desired number of layers was achieved.

**Characterizations of CuS-ST68 MBs:**

The zeta potentials of MBs during the LbL assembly of PAH and Cit-CuS NPs was determined with a PALS/90Plus Particle Sizing and Potential Analyzer (Brookhaven, Holtsville, NY, USA). A UV-vis-Near infrared photospectrometer was used to acquire the absorption spectrum of CuS-ST68 MBs. Size distributions of MBs before and
after CuS NPs modification were analyzed by static light scattering (SLS) using a Horiba LA-920 laser scattering particle size analyzer (Horiba, Tokyo, Japan). MBs samples were measured with PBS as the blank solution.

**Photothermal heating experiment:**

Different concentrations of CuS NPs dispersed in RPMI-1640 culture media were suspended in quartz cuvettes (total volume of 3.0 mL), irradiated by continuous-wave diode NIR laser (Xi’an Minghui Optoelectronic Technology, China) with a center wavelength of 808 ± 10 nm and output of 2 W for 10 min. The temperature of the solutions was measured by a digital thermometer with a thermocouple probe every 10 s. For comparative study, RPMI-1640 culture media was irradiated by NIR laser as control.

**Acoustic imaging:**

*In vitro* ultrasonography of freshly prepared ST68 MBs and CuS-ST68 MBs were carried out in the latex tube (with the inner diameter of ~5mm) using a broadband linear array L9-3 transducer (9 to 3 MHz extended) of IU22 ultrasound system (Philips Medical Systems). The same self-made setup was used for *in vitro* study as described in our previous work [Nanotechnology, 2009. 20(42): 425105.]. The MBs were diluted with 0.9% saline and injected to the latex tube stimulating the blood vessel and circulated in the tube by a constant flow pump in a permanent flow rate. Ultrasonograph was performed using the L9-3 transducer in both PIHI mode (MI=0.07) and conventional B-mode at the same time from the longitudinal cross section of the tube. The ultrasound disruption of CuS-ST68 MBs was performed under a higher ultrasound intensity (MI=0.12). After disruption, the ultrasound energy was returned to an imaging-level for diagnosis.

For *in vivo* study, three rabbits (average weight of 2.5 kg) were anesthetized with pentobarbital sodium (2.0 mL per kg weight) administration through ear vein, and subsequently, heparin sodium (4.0 mL, 0.2% w/v in 0.9% saline) was injected to avoid coagulation. The animals were placed on a warm blanket to keep body temperature within normal range during the experiment. The MBs suspension was
intravenously injected at a concentration of 0.1 mL per kg weight through a catheter, flushed with saline (1.0 mL) thereafter. The kidney was imaged trans-abdominally using a broadband L9-3 transducer in PIHI mode with MI of 0.07. The ultrasound disruption of CuS-ST68 MBs was performed under a higher ultrasound intensity (MI=0.12). After disruption, the ultrasound energy was returned to an imaging-level for diagnosis. All the digital clips and images were stored for off-line review. All the animal experiments were approved by institutional animal use committee and carried out ethically and humanely.

**In vitro photothermal tumoricidal activity of CuS-ST68 MBs:**

1 mL CuS-ST68 MBs suspension were added into 4 mL RPMI-1640 culture media and insonated for 1 min with a transmission frequency of 1 MHz and a PRF of 2 Hz using an ultrasound device (SonoPore KTAC-4000, NepaGene, Chiba, Japan). After insonation, the samples were filtered through 0.45 μm filters to simulate leaky tumor vasculature and allow only NPs to pass through. The concentrations of CuS NPs in samples before and after filtration were measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Then the filtrate of CuS-ST68 MBs was diluted in media and added to the attached cells. The filtrate of ST68 MBs were used as a control.

In this study, HeLa cells were used to evaluate the photothermal ablation efficiency with the filtrate of CuS-ST68 MBs. HeLa cells were seeded onto a 24-well plate at a density of $5 \times 10^4$ cells per well, 1 day before the experiment. The cells were washed three times with PBS, followed by incubation with 300 μL 270 μM filtrate of CuS-ST68 MBs at 37 °C and then irradiated with an NIR laser (808 nm, 6 W/cm², diameter of laser spot: 2 mm) for 0 min, 5 min and 10 min, respectively. After laser irradiation, the cells were incubated with fresh RPMI-1640 containing 10% fetal bovine serum at 37 °C for 30 min. The cells were then washed with PBS and stained with calcein AM for visualization of live cells.

We further investigated the cell survival efficiency with MTT assay. HeLa cells were seeded onto a 96-well plate at a density of 10,000 cells per well, 1 day before the
experiment. The cells were washed three times with PBS, followed by incubation with gradient concentrated filtrate of CuS-ST68 MBs at 37 °C and then irradiated with an NIR laser (808 nm, 6 W/cm², diameter of laser spot: 6.5 mm) for 0 min, 5 min. After laser irradiation, the cells were incubated with fresh RPMI-1640 containing 10% fetal bovine serum and incubated at 37 °C for 24 hrs. Cell viability was measured using the MTT assay according to the manufacturer suggested procedures.

Fig. S1 TEM image of as-prepared Cit-CuS NPs (a) and size distribution of Cit-CuS NPs (b).