Electronic Supplementary Information

Gadolinium chelates functionalized copper sulphide as a nanotheranostic agent for MR imaging and photothermal destruction of cancer cells

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Experimental Details:

Chemicals:

Diethylenetriaminepentacetic acid (DTPA, 98%, Tianjin Guangfu, China), copper(II) chloride ($CuCl_2 \cdot 2H_2O$, 98%, Tianjin Tianli, China), sodium sulfide ($Na_2S \cdot 9H_2O$, 98%, Tianjin Tianli, China), methoxy-PEG-thiol (SH-PEG; molecular weight 5000 Da, 97%, Beijing Kaizheng, China) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) were all of analytical grade and were used directly without further purification. RPMI-1640 cell culture medium (GIBCO) was supplemented with 10% fetal bovine serum, L-glutamine (0.29 mg/mL), 100 IU/mL penicillin and 100 mg/mL streptomycin. Millipore quality deionized water (DI water) (resistivity is 18.2 MU cm) was utilized in all experiments. The phosphate buffer saline (PBS) was prepared by mixing 8.010 g NaCl, 0.194 g KCl, 2.290 g $Na_2HPO_4 \cdot 12H_2O$ and 0.191 g KH_2PO_4 in 1.0 L water and the pH value was finally adjusted to 7.4.

Synthesis of CuS@DPG NPs:

The general procedure for the synthesis of CuS@DPG NPs in water was as described by Zhou et al [*J. Am. Chem. Soc*. 2010, **132**(43): 15351.] with little modifications. Briefly, DTPA (0.12 g, 0.305 mmol) was dissolved in DI water (100

mL) under stirring at 55 °C , followed by the addition of $GdCl_3 \cdot 6H_2O$ (0.038 g, 0.102 mmol) in 34.0 mL water. The molar ratio of Gd to DTPA is 1:3. After chelating 30 minutes, SH-PEG (0.03 g, 0.006 mmol) and $CuCl_2 \cdot 2H_2O$ (0.051 g, 0.300 mmol) were added to the above solution. After 10 min, $Na_2S \cdot 9H_2O$ (1mL, 0.3 M) was added into the reaction mixture. The pale-blue $CuCl_2$ solution turned dark-brown immediately upon the addition of sodium sulfide. After stirring for 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 and the CuS@DPG NPs were obtained. The CuS@DPG NPs were purified by using dialysis according to the literatures[1, 2] and stored at 4 °C for further use. The CuS@PEG-DTPA NPs that without chelating Gd[III] ions were used as control.

Characterizations of CuS@DPG NPs:

The hydrodynamic diameter (Dhy) and zeta potential of CuS@DPG NPs were determined with a PALS/90Plus Particle Sizing and Potential Analyzer (Brookhaven Instruments Co., U.S.A.). The morphology of CuS@DPG nanoparticles was observed by FEI Tecnai G2 Sphera Microscope with a CCD camera operated at 100 kV. A UV-vis-Near infrared spectrophotometer was used to acquire the absorption spectrum of CuS@DPG NPs. FTIR measurement was carried out on a Varian resolution Fourier transform infrared spectrometer (Varian FTS 3100) equipped with a wide-band MCT detector, collected with 512 background scans and 4 cm^{-1} resolution. Scanning was conducted in the mid-IR range from 400 to 4000 cm^{-1}. Samples were prepared in the forms of potassium bromide (KBr) disk. Approximately 1 mg sample and 99 mg KBr powder was blended and triturated with agate mortar and pestle. The mixture was compacted using an IR hydraulic press at a pressure of 8 tons for 1 min.

Photothermal heating experiment:

Different concentrations of CuS@DPG 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.

In vitro MR imaging:

The experiments were done using a scanner (3T MRI Achieva, Philips, Holland). For *in vitro* MR images and T_1 measurements, the CuS@DPG NPs were dispersed in aqueous solution with Gd^{3+} concentrations being 0.028, 0.045, 0.074, 0.13 and 0.173 mM respectively. The array was embedded in a phantom consisting of a tank of water to allow appropriate image acquisition. T_1 value measurements were done using an inversion recovery method with a TR of 2300 ms, a TE of 15.5 ms, and the number of signal averages of 2.

In vitro photothermal ablation of cancer cells with CuS@DPG NPs:

In this study, HeLa cells (human cervical carcinoma cell) were used to evaluate the photothermal ablation with CuS@DPG nanoparticles. For qualitative analysis, HeLa cells (6×10^4 cells per well) were incubated in 24-well plates at 37°C in a humidified atmosphere containing 5% CO_2 for 48 h. After added different concentration of CuS@DPG NPs (200 μM, 300 μM) based on copper, cells were then exposed to NIR laser (4 W/cm^2) for 0, 5 or 10 min and stained with calcein AM (calcein acetoxymethyl ester) to verify the photohyperthermic effect on cancer cells.

In vitro cytoviability assay of CuS@DPG NPs combined with NIR laser was also carried out. HeLa cells (1×10^4 cells per well) were incubated in 96-well plates at 37°C for 24 h. Different concentrationof CuS@DPG NPs (10, 20, 50, 100, 150 μM in RPMI1640) were added in. Then, the cells were irradiated by NIR laser (4 W/cm^2) for 0 min, 3 min or 5 min and the cell viability was measured using the MTT assay according to the manufacturer suggested procedures.

In vitro cytotoxicity test of CuS@DPG NPs:

Human Umbilical Vein Endothelial Cells (HUVECs) were used to represent a normal cell line in order to evaluate the cytotoxicity of CuS@DPG NPs. Cells were seeded into a 96-well cell culture plate with a density of 1×10^4 cells/well in

RPMI1640 cell culture medium under CO_2 (5%) atmosphere at 37 °C for 24 h. Then, the cells were incubated with the CuS@DPG NPs, CuS@DTPA-PEG NPs and copper chloride with different concentrations based on copper (10, 50, 100, 200, 500, 1000 μM in RPMI1640) for 24 h at 37 °C under 5% CO_2. Thereafter, MTT (20 μL, 5 mg/mL) was added to each well and the plate was incubated for 4 h at 37 °C. After the addition of dimethyl sulfoxide (DMSO, 150 μL/well), Wells were then aspirated and 150 μL dimethylsulfoxide (DMSO) was added to each well to dissolve the dark blue crystals thoroughly. The absorbance was measured at 490 nm using a microplate reader (Thermo, Multiskan MK3). The IC50 values (i.e., concentration resulting in 50% growth inhibition) were calculated by the curve fitting of the cell viability data, considering the optical density of the control well as 100%.

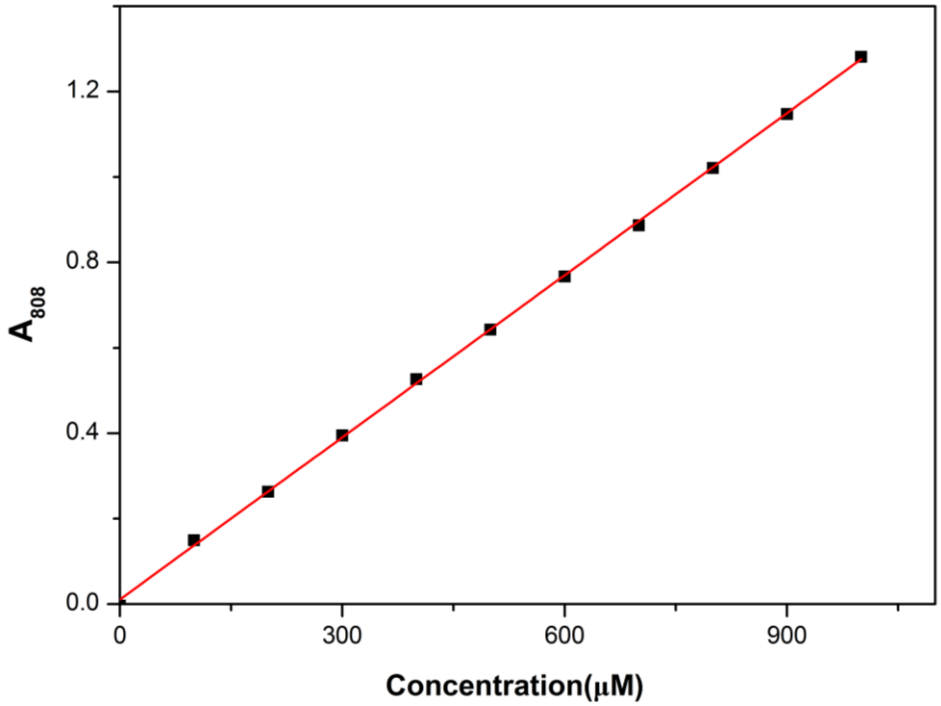

Fig. S1 Fitting curve of the UV absorption of CuS@DPG NPs at different concentrations in the medium at 808 nm (R^2=0.9997).

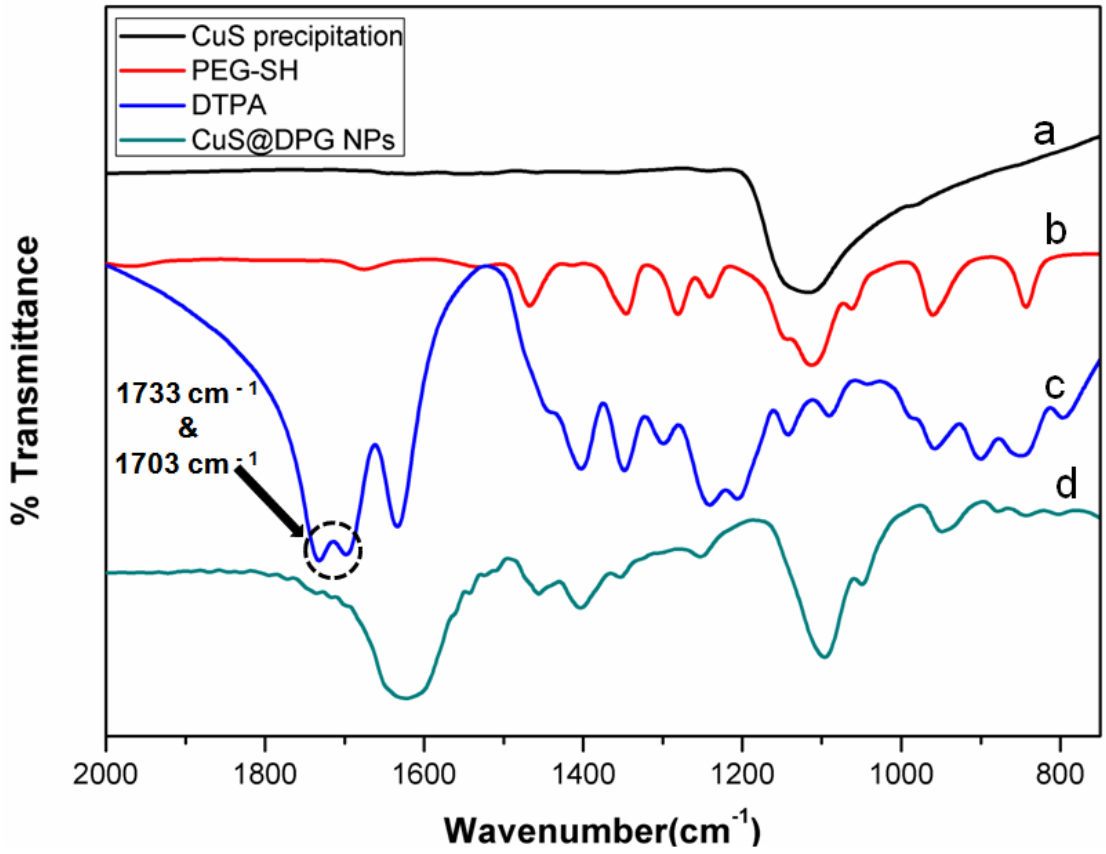

Fig. S2 FTIR spectra of a) CuS precipitation, b) PEG-SH, c) DTPA and d) CuS@DPG NPs.

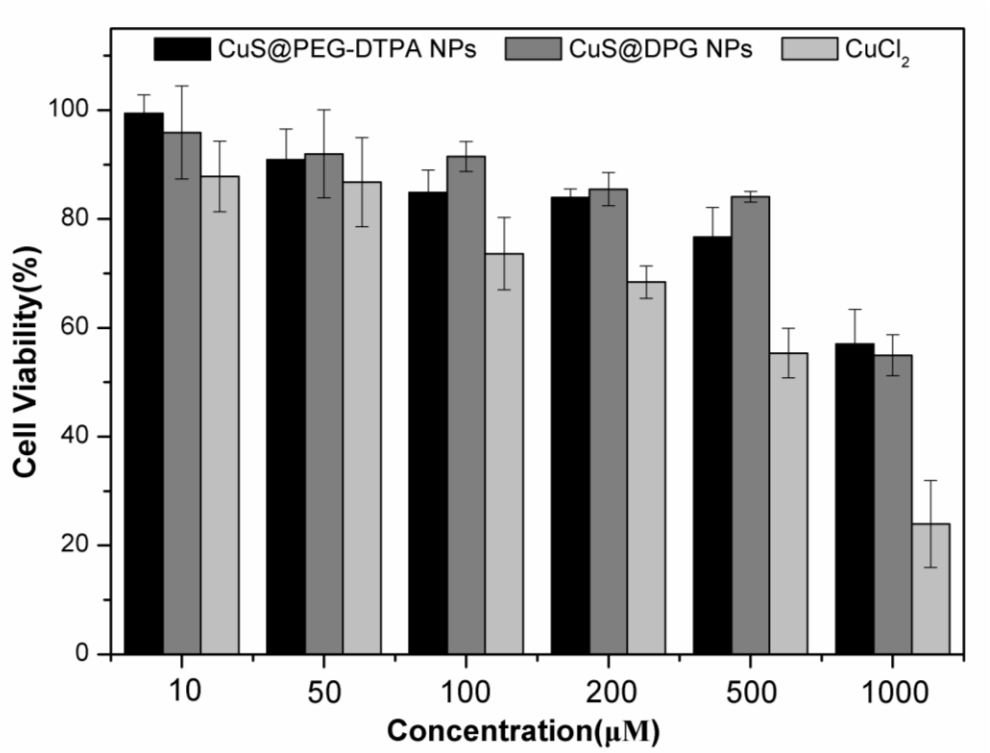

Fig. S3 Cytotoxicity of CuS@PEG-DTPA NPs, CuS@DPG NPs and $CuCl_2$ aqueous solution in HUVE cells in HUVE cells. The cells were incubated in culture medium containing NPs at concentrations ranging from 10μM to 1 mM for 24 h.

References

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2. K. H. Bae, Y. B. Kim, Y. Lee, J. Hwang, H. Park and T. G. Park, *Bioconjugate Chem*, 2010, **21**, 505-512.