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Supporting Information

A Cu^{2+} doped mesoporous polydopamine Fenton nanoplatform for low-temperature photothermal therapy

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EXPERIMENTAL SECTION

Materials

Copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), H_2O_2, DMSO, ethanol and acetone were purchased form Sinopharm Chemical Reagent Co., Ltd. Dopamine hydrochloride, β-Lapachone (Lapa) and coumarin were purchased from Energy Chemical Co., Ltd (Shanghai, China). F127 and 3-(4,5-dimethyl-thiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) were purchased from Sigma (Shanghai, China). TRIS was purchased from Beijing Solarbio Science &Technology Co., Ltd. Hyaluronic acid (HA) was purchased from Shandong Freda Biotechnology Co., Ltd. Methylene blue (MB) were purchased from Macklin Biochemical Co. Ltd (Shanghai, China). DTNB was obtained from Cayman Chemical (USA); 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) was obtained from Beyotime Biotechnology (China); Calcein-AM/PI double staining kit was obtained from Yeasen Biotech Co., Ltd (Shanghai, China). Confocal dish was purchased from Cellvis, Mountain View, CA. The chemical reagents used in the experiment were analytical grade and without further purification. Penicillin/streptomycin were purchased from Gibco. The mouse breast cancer cell line (4T1) was purchased from KeyGEN biotechnology (Nanjing, China). The experimental water used was Mill-Q secondary ultrapure water (18.2 $M\Omega \cdot cm^{-1}$).

Instruments

Transmission electron microscopy (TEM) was carried out on a JEM-100CX II electron microscope. Dynamic light scattering and zeta potential measurements were conducted with Malvern Zeta Sizer Nano (Malvern Instruments). X-ray photoelectron spectroscopy (XPS) were carried out using an Escalab 250Xi instrument (Thermo Scientific, USA). UV-Vis absorption spectra were measured on pharmaspec UV-1700 UV Visible spectrophotometer (Shimadzu, Japan). MTT assay was performed in a microplate reader (Synergy 2, Biotek, USA). Confocal fluorescence imaging assay were performed with a TCS SP8 confocal laser scanning microscopy (Leica, Germany). Live animal imaging system (IVIS Lumina III, US) was applied *in vivo* imaging.

Hydroxyl radical (·OH) generation assay

Coumarin (0.5 mM), H_2O_2 (0.2 mM) and CuPDA (0.1 mg) was mixed in 1 mL PBS buffer. After stirred for 1 h in the dark, the solution was irradiated with 808 nm laser (1.0 W/cm^2) for 10 min. NPs were removed by centrifugation and the fluorescence intensity was measured.

H_2O_2 (0.2 mM) and CuPDA (0.1 mg) was mixed in PBS buffer and then irradiated with 808 nm laser (1.0 W/cm^2) for 10 min. 2,2,6,6-tetramethylpiperidine (TEMPO) solution (6.0 mg/mL) was added into it and ESR spectra were measured.

Cellular uptake

Cy5 was loaded into the nanoparticles as the fluorescent group. 4T1 cells were divided into three groups (PBS, CuPDA-Cy5, CuPDA-Cy5@HA) and seeded onto glass bottom dishes (20 mm) for 24 h. After incubated with nanoparticles respectively for 1 h, nanoparticles were removed and washed with PBS for three times. Then cells were stained with Hoechst for 15 min. Cellular uptake was observed by confocal fluorescent imaging.

Cytotoxicity assay

The cytotoxicity of nanoparticles was evaluated by methyl thiazolyl tetrazolium (MTT) assay. The 4T1 cells were planted into 96 well plate for 24 h, and incubated with different concentrations (0, 0.02, 0.05, 0.10, 0.20, 0.50 mg/mL) of PDA@HA, CuPDA@HA and CuPDA-Lapa@HA. After incubated for another 12 h, the nanoparticles were removed and washed with PBS for three times. Then 200 μL MTT (0.5 mg/mL) was added into the 96 well plate for 4 h incubation and DMSO was then used to dissolve formazan crystal. The absorbance at 490 nm was measured with the RT 6000 microplate reader.

The cytotoxicity of nanoparticles was evaluated by methyl thiazolyl tetrazolium (MTT) assay. The TC-1, MCF-10a and HL-7702 cells were respectively planted into 96 well plate for 24 h, and incubated with different concentrations (0, 0.02, 0.05, 0.10, 0.20, 0.50 μg/mL) of CuPDA-Lapa@HA. After incubated for another 12 h, the nanoparticles were removed and washed with PBS for three times. Then 200 μL MTT (0.5 mg/mL) was added into the 96 well plate for 4 h incubation and DMSO was then

used to dissolve formazan crystal. The absorbance at 490 nm was measured with the RT 6000 microplate reader.

***In vitro* therapeutic conditions optimizing**

The 4T1 cells were planted into 96 well plate for 24 h, and then incubated with 0.10 mg/mL CuPDA-Lapa@HA for 4 h, then were irradiated under 808 nm laser with each power density (0.5, 1.0, 1.5 W/cm^2) for different times (2, 4, 6, 8, 10 min). After incubated for another 6 h, 200 μL MTT (0.5 mg/mL) was added into the 96 well plate for 4 h incubation and DMSO was then used to dissolve formazan crystal. The absorbance at 490 nm was measured with the RT 6000 microplate reader.

Photothermal cytotoxicity

The 4T1 cells were planted into two 96 well plates for 24 h, and divided into 6 groups: control, only laser, PDA@HA, CuPDA@HA, CuPDA-Lapa@HA and CuPDA-Lapa@HA with laser. After incubated with 0.1 mg/mL nanoparticles for 4 h, the groups needed laser were irradiated under 808 nm (1.0 W/cm^2) laser for 10 min. After incubated for another 6 h, 200 μL MTT (0.5 mg/mL) was added into 96 well plate for 4 h incubation and DMSO was then used to dissolve formazan crystal. The absorbance at 490 nm was measured with the RT 6000 microplate reader.

***In vivo* photothermal imaging**

Balb/C mice were intravenously injected with 0.3 mg of CuPDA-Lapa@HA dispersed in 100 μL saline. The tumor was irradiated with 808 nm laser (1.0 W/cm^2) for 10 min at different times (3, 6, 9, 12, 24 h) after injection. Photothermal imaging of mice were recorded by the IR imaging devices.

Ex vivo fluorescent imaging

To investigate the biological distribution of nanoparticles in tumors and organs, the Cy5-labeled nanoparticles were employed for fluorescent imaging. Balb/C mice were intravenously injected with 0.3 mg of CuPDA-Cy5@HA dispersed in 100 μL saline. Then the mice were sacrificed at 12 h after injection and the tumor and organs were removed for fluorescent imaging.

Supporting Figures

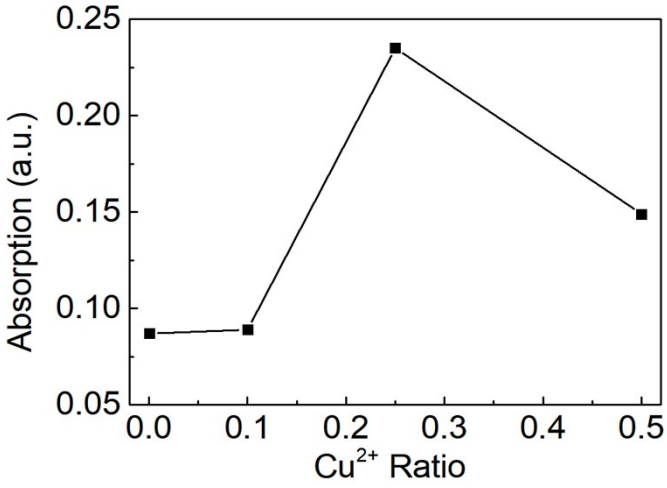


Figure S1. The absorption at 808 nm of CuPDA with different Cu^{2+} ratio.

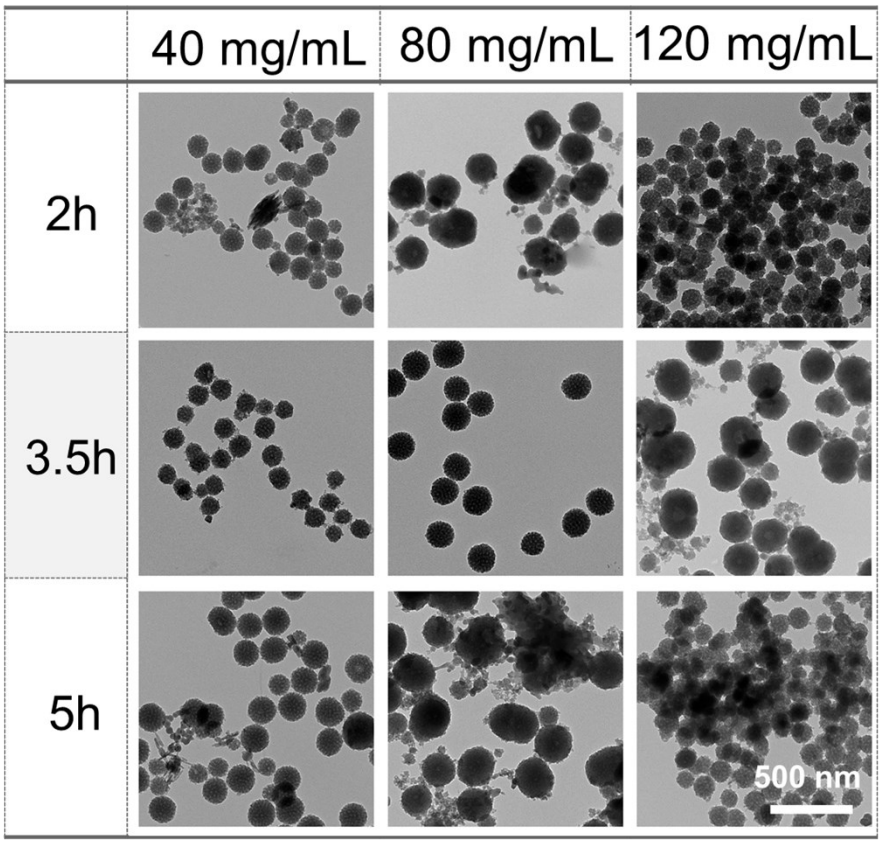


Figure S2. The TEM images of CuPDA NPs with different reaction time or different Tris concentration.

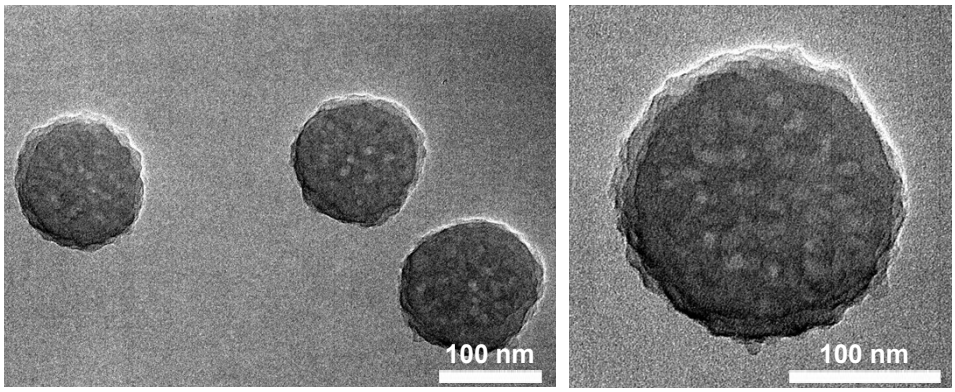


Figure S3. The HRTEM images of CuPDA NPs with the reaction time of 3.5 h and the Tris concentration of 80 mg/mL.

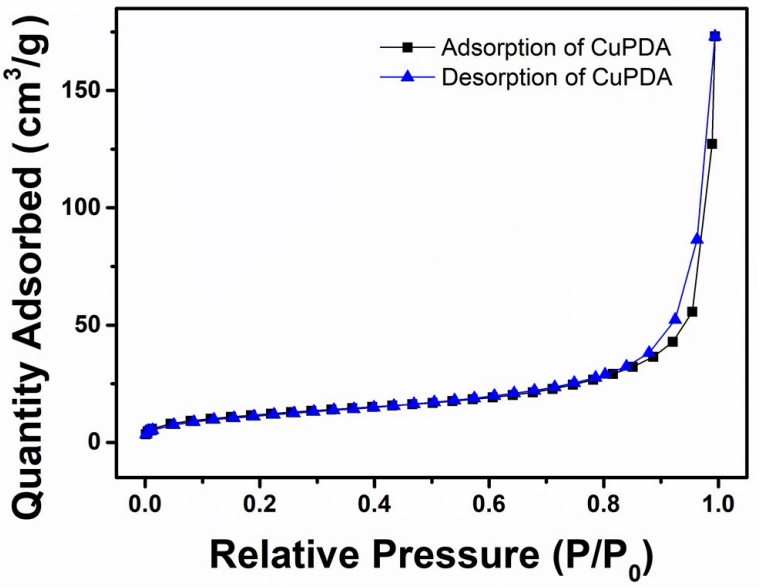
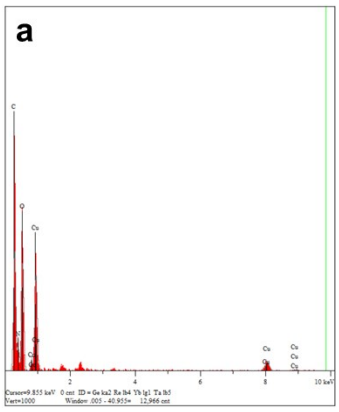


Figure S4. N_2 adsorption-desorption isotherms of CuPDA NPs and the pore size distribution of CuPDA NPs (inset).

a



b

Elt.	Line	Intensity (c/s)	Conc	Units
C	Ka	60.52	29.597	wt.%
N	Ka	9.92	14.409	wt.%
O	Ka	51.12	38.647	wt.%
Cl	Ka	.95	.231	wt.%
Cu	Ka	8.19	17.116	wt.%
			100.000	wt.%

Figure S5. The EDX spectra of CuPDA and the corresponding mass ratio of different elements.

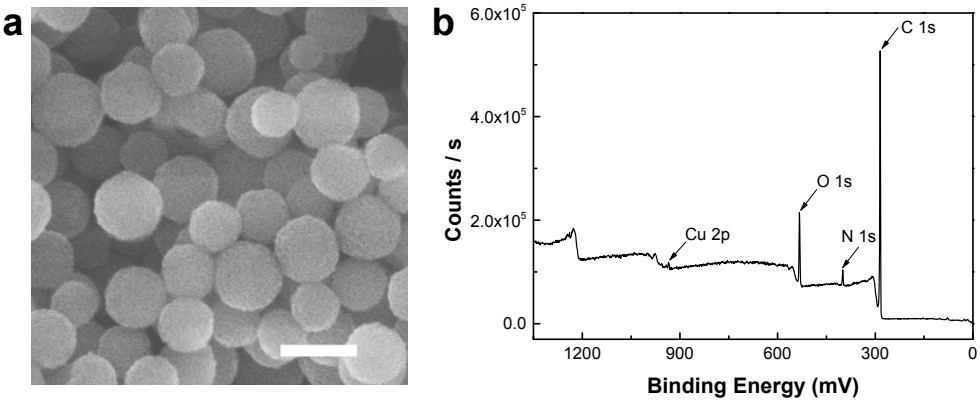


Figure S6. (a) The SEM images of CuPDA; (b) The XPS spectra of CuPDA.

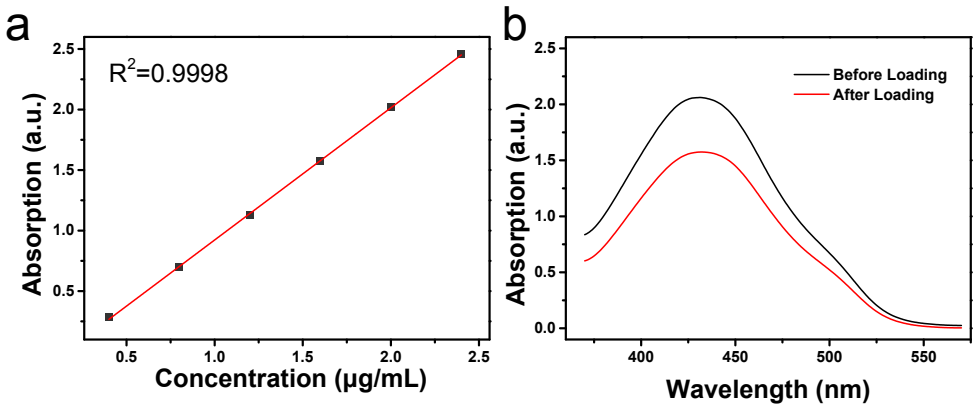


Figure S7. (a) The standard curve for absorbance values of Lapa by nanodrop. (b) Absorption spectra of Lapa before and after loading.

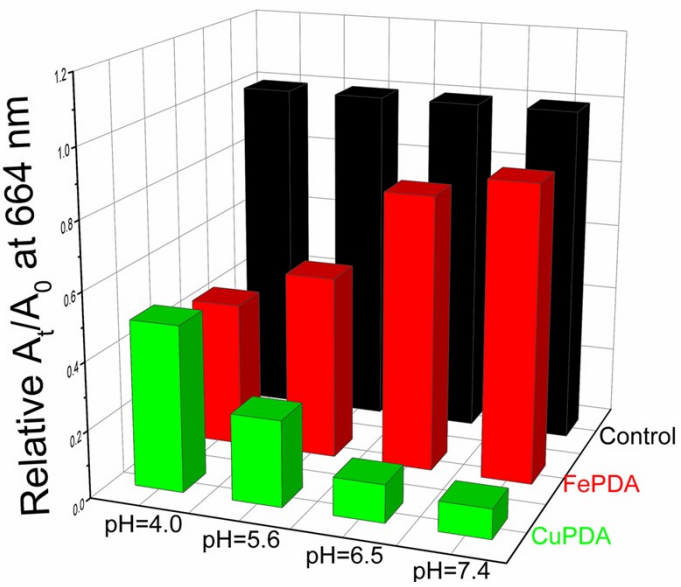


Figure S8. MB degradation in CuPDA or FePDA solution at different pH buffer solution.

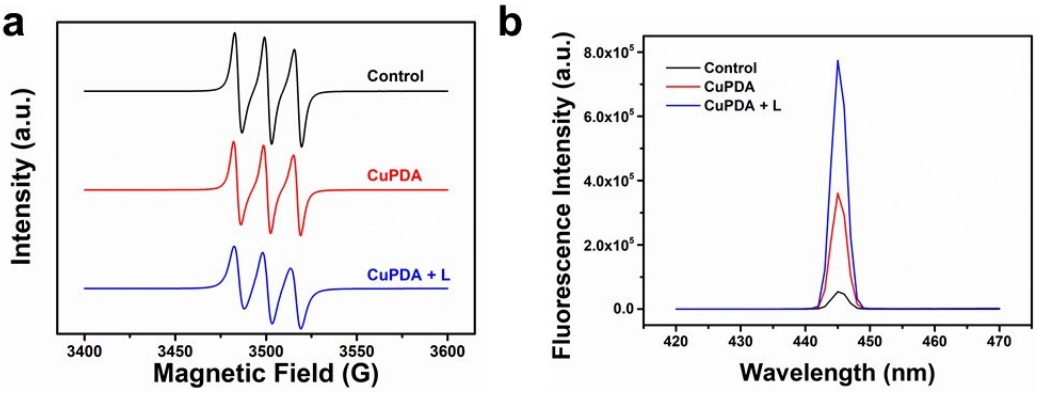


Figure S9. The detection of ·OH generation. (a) ESR spectra of TEMPO in solution after incubation with PBS, CuPDA, and CuPDA+L. (b) The fluorescence spectra of coumarin after incubation with PBS, CuPDA, and CuPDA+L. ("L" refers to the irradiation with 808 nm laser (1.0 W/cm^2) for 10 min.

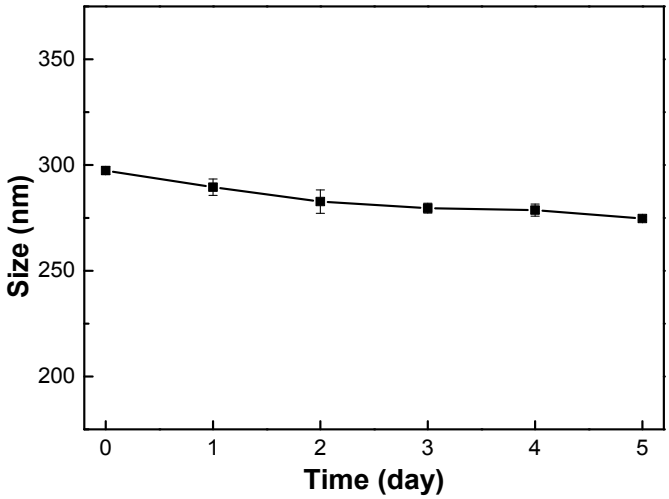


Figure S10. The particle diameter of CuPDA@HA measured by DLS analysis at different time.

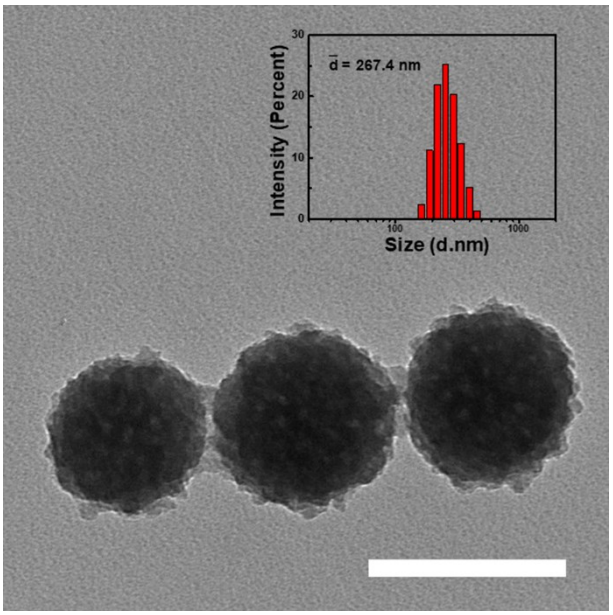


Figure S11. The TEM image and DLS analysis of PDA@HA.

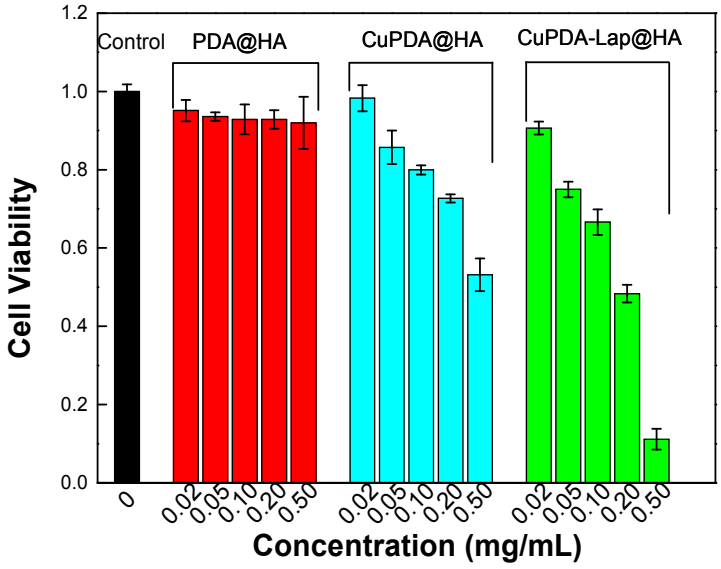


Figure S12. Viability of 4T1 cells incubated with different nanomaterials and different concentrations for 12 h.

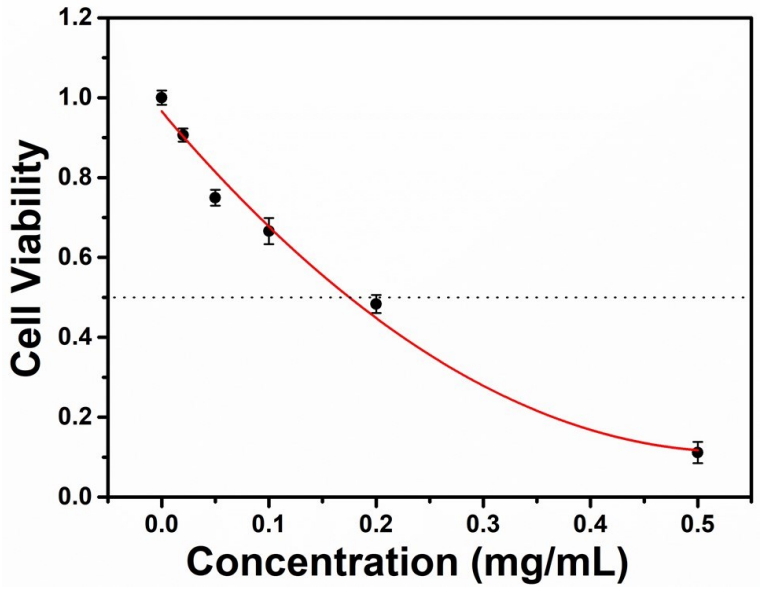


Figure S13. The measurement of IC50 value of CuPDA-Lapa@HA NPs *via* MTT assay.

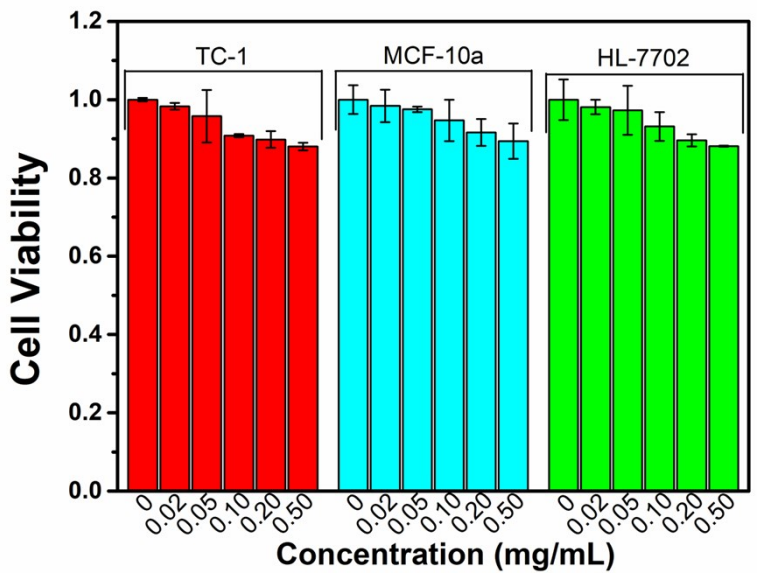


Figure S14. Cell viability of normal cells incubated with different nanomaterials and different concentrations of CuPDA-Lapa@HA NPs for 12 h.

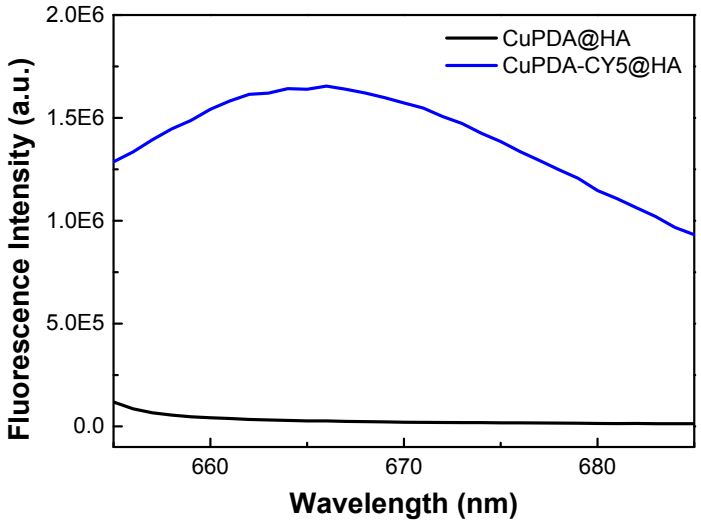


Figure S15. The florescence intensity in CuPDA@HA solution or in CuPDA-Cy5@HA solution.

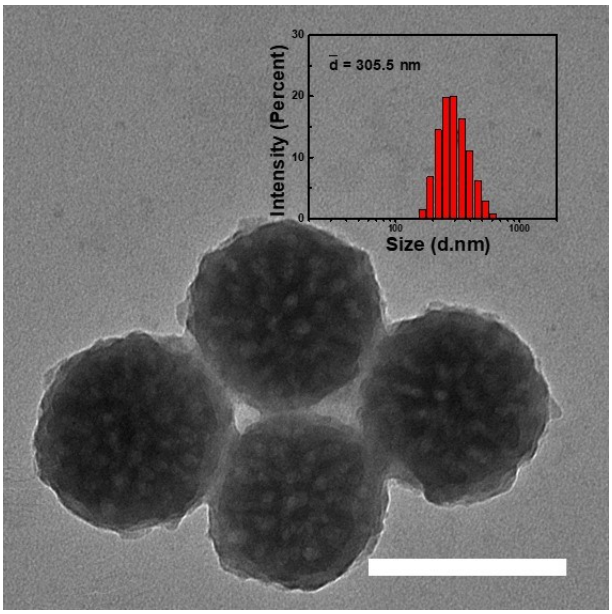


Figure S16. The TEM image and DLS analysis of CuPDA-Cy5@HA.

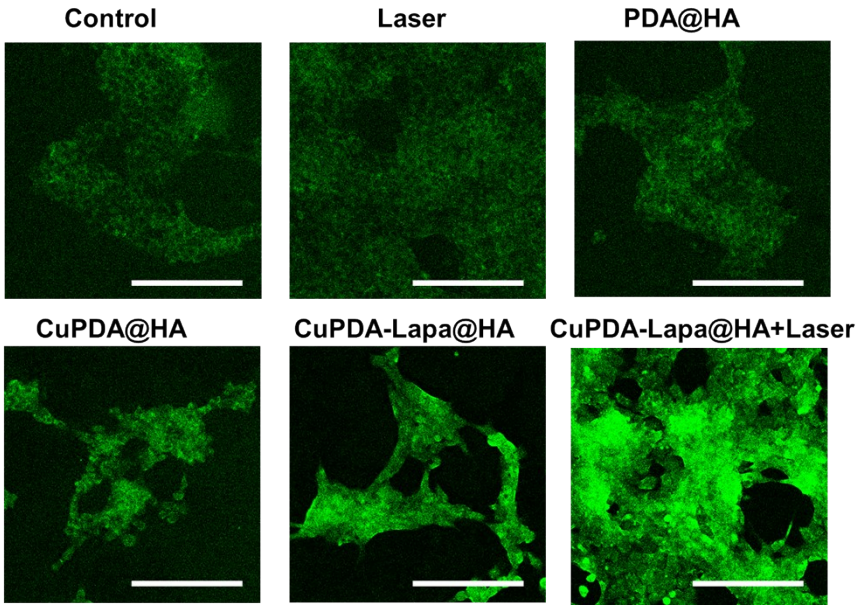


Figure S17. Confocal images of coumarin stained 4T1 cells in different group. Scale bar = 200 μm.

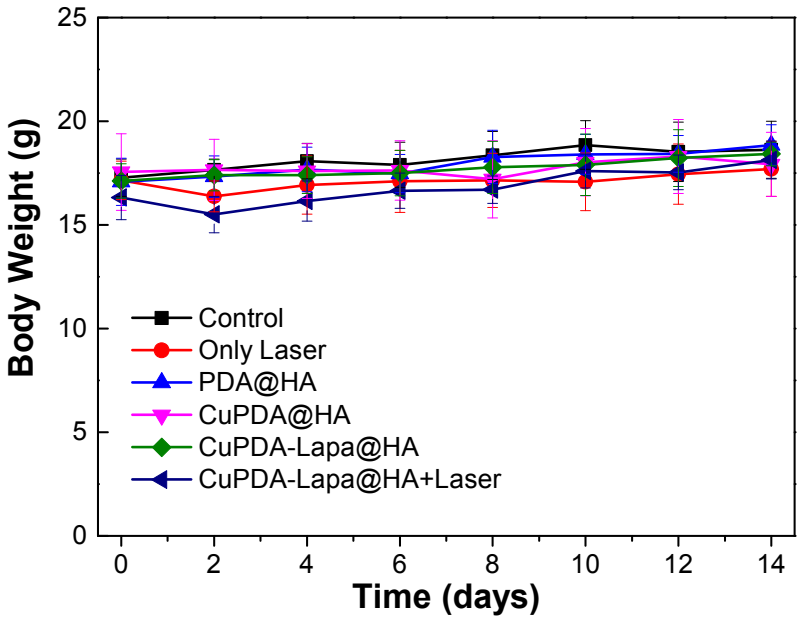


Figure S18. Mice body weight curves in different treatment groups.

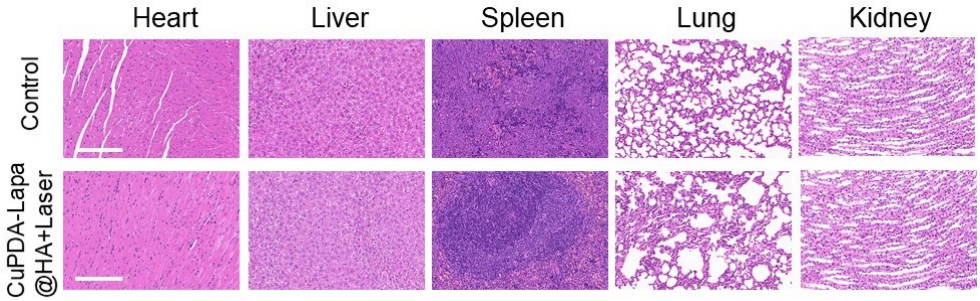


Figure S19. H&E staining of the five major organs (heart, liver, spleen, lung and kidney) with different treatments after 12 h. Scale bars are 200 μm.

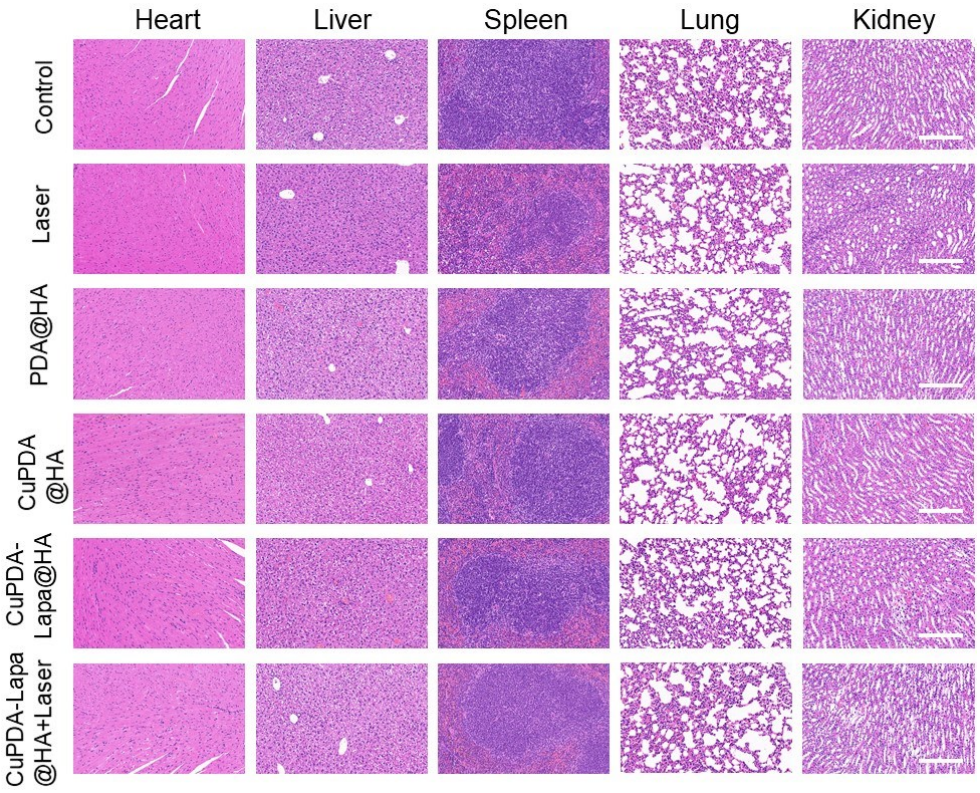


Figure S20. H&E staining of the five major organs (heart, liver, spleen, lung and kidney) with different treatments after 7 days. Scale bars are 200 μm.