Electronic Supplementary Information

Multifunctional and Flexible ZrO_2-Coated EGaIn Nanoparticles for Photothermal Therapy

Na Xia,[a,b] Na Li,[c] Wei Rao,*[b] Jie Yu,[d] Qiong Wu,[b] Longfei Tan,[b] Hongbo Li,[e] Li Gou,*[a] Ping Liang,[d] Laifeng Li [b] and Xianwei Meng *[b]

[a] College of Materials Science and Engineering, Sichuan University, Chengdu 610065 China

[b] Laboratory of Controllable Preparation and Application of Nanomaterials, CAS Key, Laboratory of Cryogenics, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, 29 Zhongguancun East Road, Beijing 100190 China

[c] The 304 Research Institute of China Aerospace Science and Industry Corp

[d] Department of Interventional Ultrasound, Chinese PLA General Hospital, Beijing 100853 China

[e] Beijing Key Laboratory of Construction-Tailorable Advanced Functional Materials and Green Applications, School of Materials Science & Engineering, Beijing Institute of Technology, Beijing 10081 China

Experimental

Reagents: Liquid metal (75.5% gallium and 24.5% indium, by weight). Anhydrous ethanol and acetonitrile were supplied by Beijing Chemical Plant. Sorbitol trioleate (Span 85) was provided by Shanghai Macleans Biochemical Technology Co., Ltd. Zirconium n-propoxide was purchased from Tokyo Chemical Industry Co., Ltd. Ammonia was supplied by Sinopharm Group Chemical Reagent Co., Ltd. Polyethylene glycol-decyl (PEG-SH) was purchased from Beijing Kaizheng United Pharmaceutical Technology Co., Ltd.

Synthesis of LM@pZrO_2 NPs: LM@pZrO_2 NPs were synthesized by a two-step method. The first step was to synthesize LM@ZrO_2 NPs. 0.15mL LM was dispersed evenly in a mixture of 60 mL ethanol and 0.03 mL span 85 by ultrasound. Then 20 mL acetonitrile and 0.15 mL ammonia were immersed in the mixed solution to form A mixture. Meanwhile, 5 mL acetonitrile and 0.063 mL zirconium n-propoxide were immersed to 15 mL ethanol to form B mixture. Finally, B mixture was stirred in A mixture with a magnetic stirrer at room temperature for 12 h. LM@ZrO_2 NPs were obtained by centrifuging and washing with ethanol and water. The second step was to synthesize LM@pZrO_2 NPs. 20 mg obtained LM@ZrO_2 NPs were dispersed in 10 mL deionized water, then 10 mg pSH_{5000} was dispersed and stirred in the solution for 2 h at room temperature. After centrifuging and washing with water, LM@pZrO_2 NPs were synthesized.

Characterization: The morphology and particle size of LM@ZrO_2 NPs were measured by scanning electron microscopy (SEM, Model 4300, Hitachi, Japan) and transmission electron microscopy (TEM, HT7700, Hitachi, Japan). The element type and distribution were characterized by SEM and energy dispersive spectrometer (EDS). Laser particle size analyzer (Malvern instruments Zeta-sizer Nano ZS90, Britain) was employed to characterize surface potential change. The characteristic absorption spectrum was confirmed by an ultraviolet spectrophotometer (UV-Vis, Jasco V-570 UV/vis/NIR). The heat distribution was measured by a near infrared imager. Scanning probe microscope (Bruker Multimode 8, Veco, America) was employed to confirm young's modulus. The cell viability was analyzed by a microplate reader. Fluorescence microscopy (Olympus X71, Japan) was used to observe HE stained cells. Internalization of materials was analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Varian 710-OES, America).

Stability experiment: 3 mg LM@ZrO_2 NPs and LM NPs were dispersed in 1.5 ml water, respectively, and then stationed to verify the dispersing stability in water. Next, LM NPs and LM@ZrO_2 NPs were dispersed in water. The morphology of LM NPs and LM@ZrO_2 NPs at different time was observed by transmission electron microscopy, and the hydrated particle size was measured by laser particle size analyzer.

Young's modulus test: The mechanical properties of the material surface were characterized by measuring the young's mode *via* scanning probe microscope. The calibrated Bruker probe had a cantilever spring constant of 6.432 N m^{-1} and a tip radius of 99 nm. The materials were dispersed in ethanol. After ultrasound for 30 min, a drop of the suspension was placed on a freshly cleaned mica sheet surface. Measurement of mechanical properties after natural evaporation of solvents. The young's modulus was the average modulus values of three different small regions in the modulus map.

Cytotoxicity experiment: MTT method was used to detect the cytotoxicity of the material. Using HepG2 cells as cell models, cells in logarithmic phase were collected, and 100 μL cell suspension was added to each hole of 96-well plate to make the cell density to 1000-10000 each hole. The cells were incubated at 5 % CO_2 and 37 °C until the cell monolayer covered the bottom of the pore. Then 100 μL LM@pZrO_2 was added to the well plates to give material concentrations of 0, 12.5, 25, 50, 75, 100, 200 and 400 μg mL^{-1}, with 5 parallel tests for each concentration. After

24 h of incubation, 20 μL MTT solution (5 mg ml^{-1}, 0.5% MTT) was added to each pore. 4 hours later, the culture medium was poured out and 150 μL dimethyl sulfoxide was added. The obtained suspension was centrifuged at 10000 r for 10 min to obtain 120 μL supernatant. And the absorbance of each well was measured at OD 492 nm by elisa reader. Cell activity was calculated by absorbance value.

Internalization of materials: 50 μg $pZrO_2$ NPs, pLM NPs and LM@$pZrO_2$ NPs were dispersed in 1 mL DMEM, respectively. HepG2 cells were incubated with these materials for 8 h. The materials not internalized by the cells were washed away with PBS, and the cells were digested with trypsin to digest (digested solution A). Meanwhile, 100 μg these materials were digested (digested solution B). All digested solutions were tested for ICP-MS. Ratio of internalized materials = Ga or Zr ion concentration of digested solution A / Ga or Zr ion concentration of digested solution B × 2 × 100 %.

PT experiment: PT conversion performance of LM@ZrO_2 NPs were measured by PT experiment. 100, 300 and 500 μg LM@ZrO_2 NPs were dispersed in 1 mL H_2O, respectively, and irradiated with 808 nm NIR laser light (1.8 W) for 5 minutes. A near infrared imager was employed to record the temperature distribution and variation on the surface of the solution.

Cell therapy experiment: The HepG2 cells covered with the bottom of the 6-well plate were incubated with LM@$pZrO_2$ NPs with different concentrations (0, 50, 100 and 200 μg mL^{-1}, dispersed in DMEM) for 12 h. The surface of the cells was washed with PBS, and the cells in the 6-well plate were digested with trypsin and added to a 96-well plate. Each group was set with 5 small holes in a 96-well plate. The NIR laser pointer was aimed at the small holes, and the cells of each hole was irradiated for 5 min at 1.8 W. After 24 h of incubation, 20 μL MTT solution was added to each pore. 4 hours later, the culture medium was poured out and 150 μL dimethyl sulfoxide was added. The obtained suspension was centrifuged at 10000 r for 10 min to obtain 120 μL supernatant. And the absorbance of each well was measured at OD 492 nm by elisa reader. Cell activity was calculated by absorbance value. Similarly, the HepG2 cells covered with the bottom of the 6-well plate were incubated with 200 μg mL^{-1} LM@$pZrO_2$ NPs for 12 h. After washed with PBS, HepG2 cells were digested and added to DMEM in 96-well plates, divided into four groups, each group with 5 wells in 96-well plates, and other four groups were not incubated with LM@$pZrO_2$ NPs, which were used as the control group. The NIR laser pointer was aimed at the hole. Each group was irradiated for different time (0, 3 and 6 min) at 1.8 W. The cells were subsequently treated as described previously and tested for cell viability.

The live/dead cells staining: HepG2 cells were incubated with 200 μg mL^{-1} LM@$pZrO_2$ for 12 h, and then irradiated with NIR irradiation for 5 min at 1.8 W. After 24 hours of culture, the cells were digested and washed three times. The cells were incubated with Calcein-AM and PI for 15 minutes. Cell fluorescence was characterized by confocal fluorescence microscopy.

CT imaging experiment: 1, 4, 8, 12, 16 and 20 mg LM@ZrO_2 NPs were dispersed in 1 ml of water for *in vitro* CT testing. CT imaging experiment *in vivo* was performed at 0, 3, 6, and 9 h after injection of 50 mg kg^{-1} LM@$pZrO_2$ into the tail vein of a mouse, and the CT value of the tumor site was recorded.

Anti-tumor experiment in vivo: The mice with a body weight about 28 g was used for anti-tumor experiment *in vivo*. The mice were divided into four groups (control group, LM@$pZrO_2$ group, PT group and LM@$pZrO_2$ + PT group) with three mice in each group. Mice in the LM@$pZrO_2$ group and the LM@$pZrO_2$ + PT group were injected with 50 mg kg^{-1} LM@$pZrO_2$ *via* the tail vein. After the injection for 6 h, mice in the LM@$pZrO_2$ + PT group and the PT group were subjected to PTT, and the tumor site was irradiated with 808 nm NIR for 5 min at 1.8 W. This process was

recorded by a near infrared imager. The mice were fed for 2 weeks. The weight of mice was recorded and the volume of tumors was measured. Then the mice were executed and the tumor, heart, liver, spleen, lung and kidney were collected. The weight of the tumors was measured. After paraffin immobilization and HE staining, the tissue sections were observed by optical microscopy.

Toxicity experiment: The mice with a body weight about 28 g was used for the material toxicity test. Mice were injected with different doses (0, 50, 100, 150 and 200 mg kg^{-1}, dispersed in DMEM) of LM@pZrO_2 NPs *via* tail vein, and each dose with three mice. The activity of the mice was observed and the body weight of the mice was recorded. Two weeks later, the mice were executed and their blood was collected to test blood routine and biochemistry. The heart, liver, spleen, lung and kidney of the mice were collected, and after paraffin fixation and HE staining, the tissue sections were observed with an optical microscope.

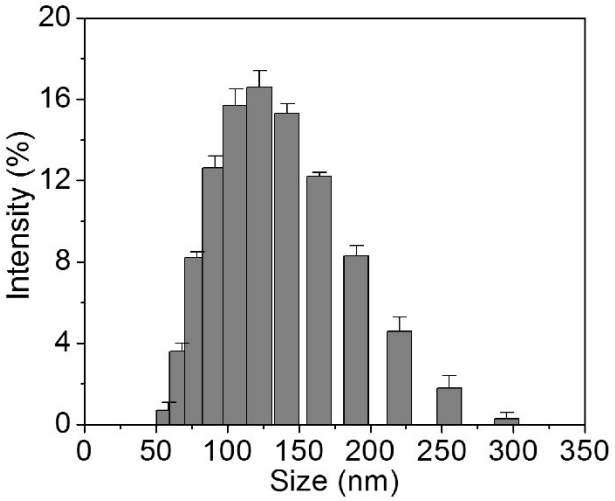

Figure S1. Hydrodynamic diameter of LM@ZrO_2 NPs.

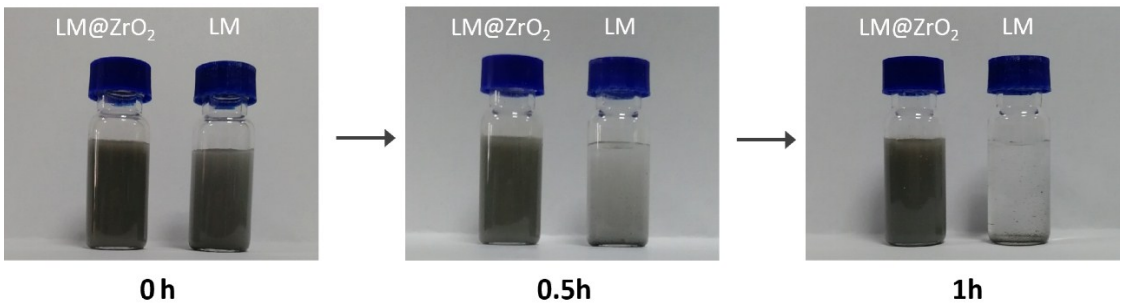

Figure S2. Dispersion stabilities of LM@ZrO_2 NPs and LM NPs in water.

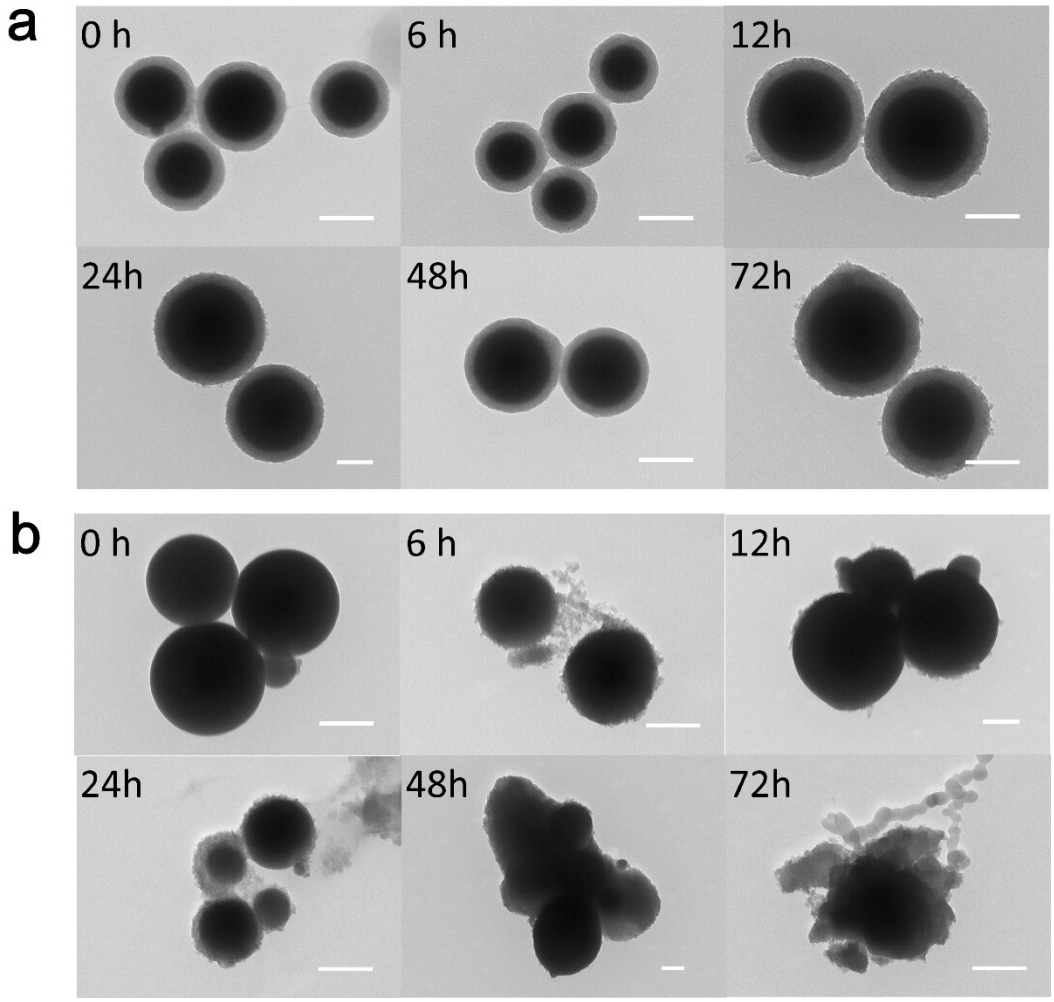

Figure S3. TEM images of LM@ZrO_2 NPs and LM NPs dispersed in water under different time intervals.

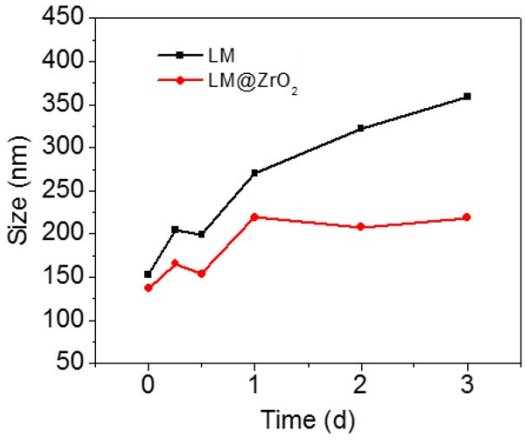

Figure S4. Hydration particle size of LM@ZrO_2 NPs and LM NPs dispersed in water under different time intervals.

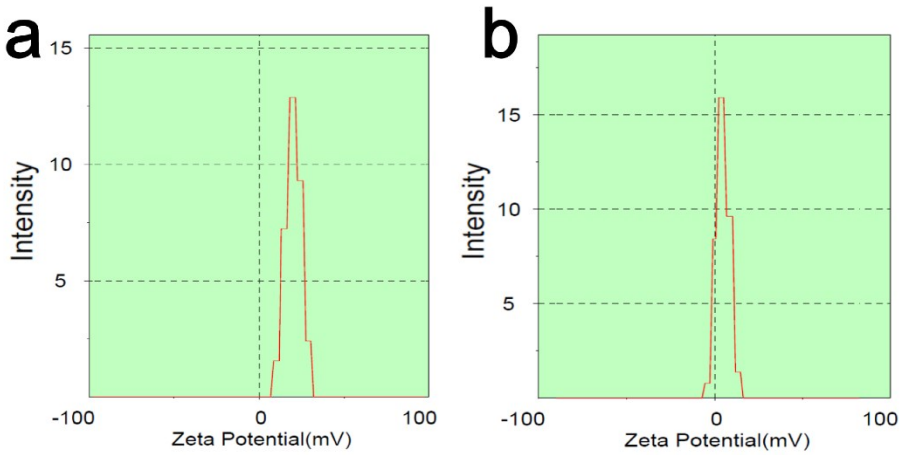

Figure S5. a) and b) were zeta potential diagram of LM@ZrO_2 NPs and LM@pZrO_2 NPs.

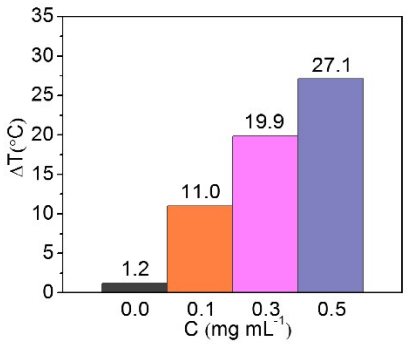

Figure S6. Temperature change histogram of the solution dispersed LM@ZrO_2 NPs.

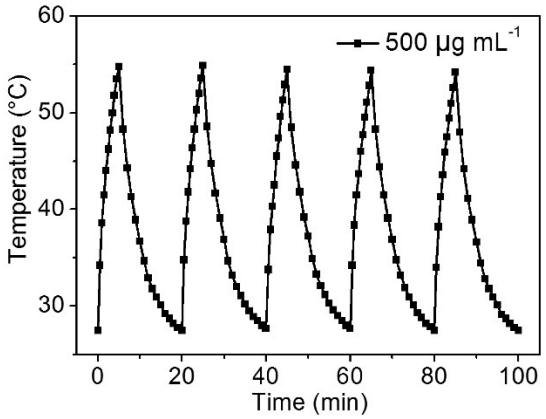

Figure S7. Repeat NIR laser irradiation for LM@ZrO_2 NCs aqueous solution.

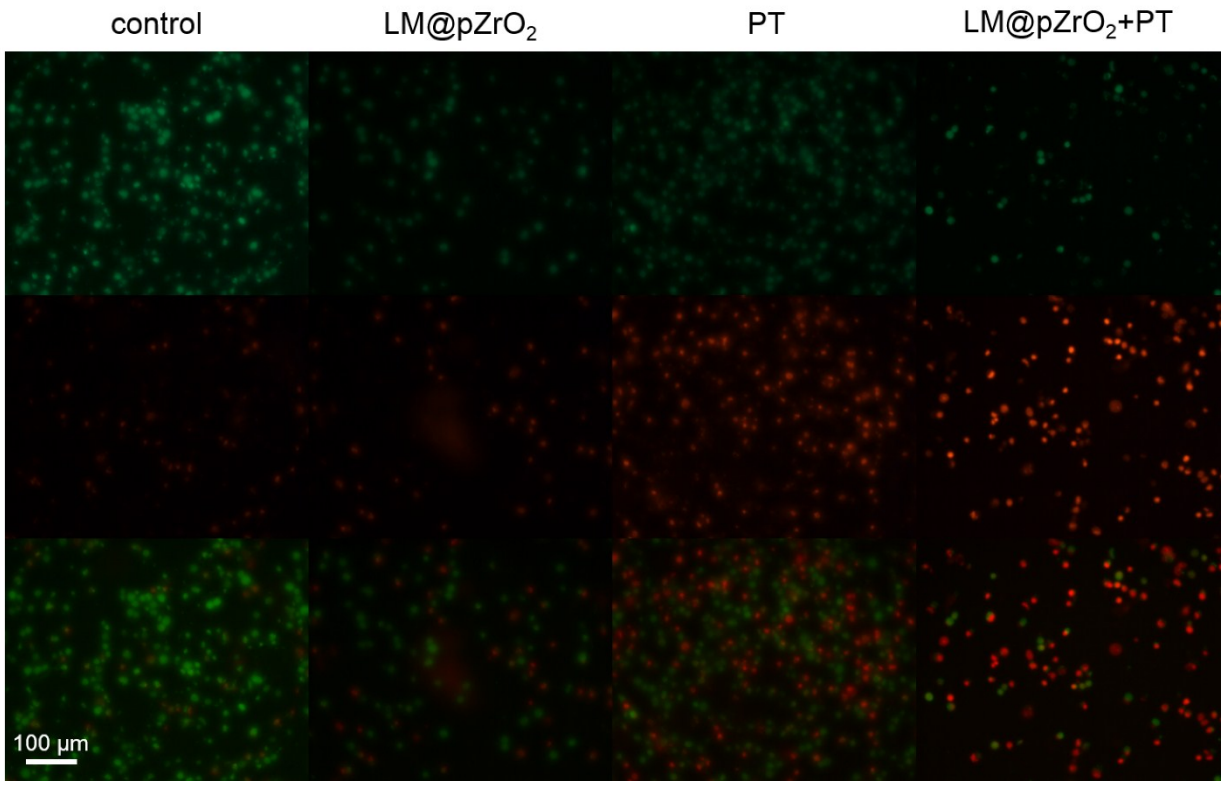

Figure S8. Confocal fluorescence images of HepG2 cells after the live/dead cells staining.

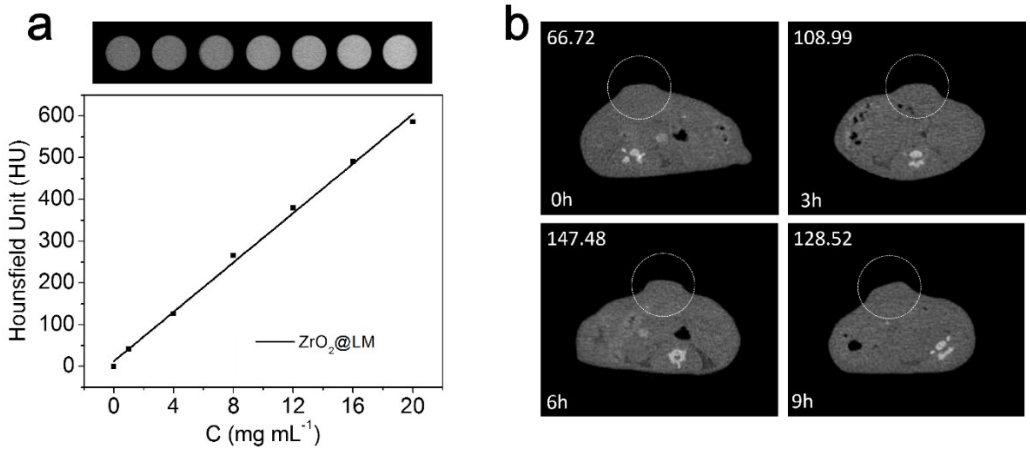

Figure S9. a) CT imaging and value of LM@ZrO_2 NPs with different concentrations (1, 4, 8, 12, 16 and 20 mg mL^{-1}). b) CT imaging of tumor location in mice at 0, 3, 6 and 9 h after injection of 50 mg kg^{-1} LM@pZrO_2 NPs *via* tail vein.

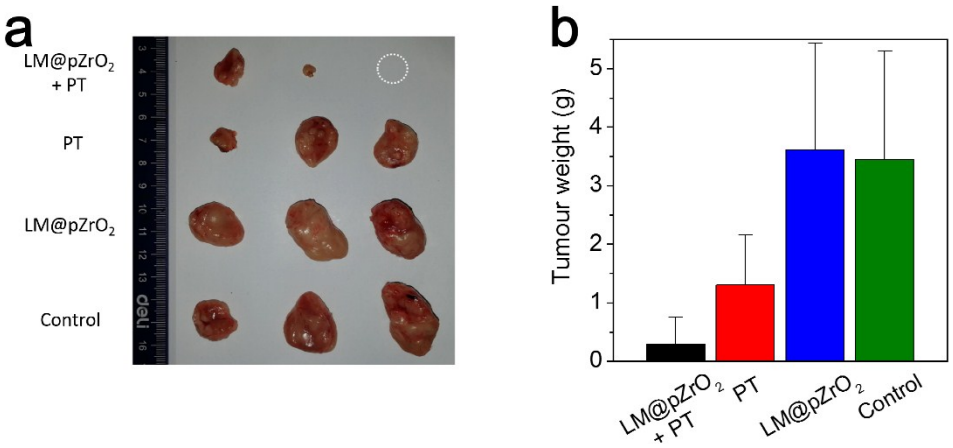

Figure S10. a) Photographs of tumors taken from mice in different groups (control group, LM@$pZrO_2$ group, PT group and LM@$pZrO_2$ + PT group) after being executed. b) Histogram of tumor weight based on a).

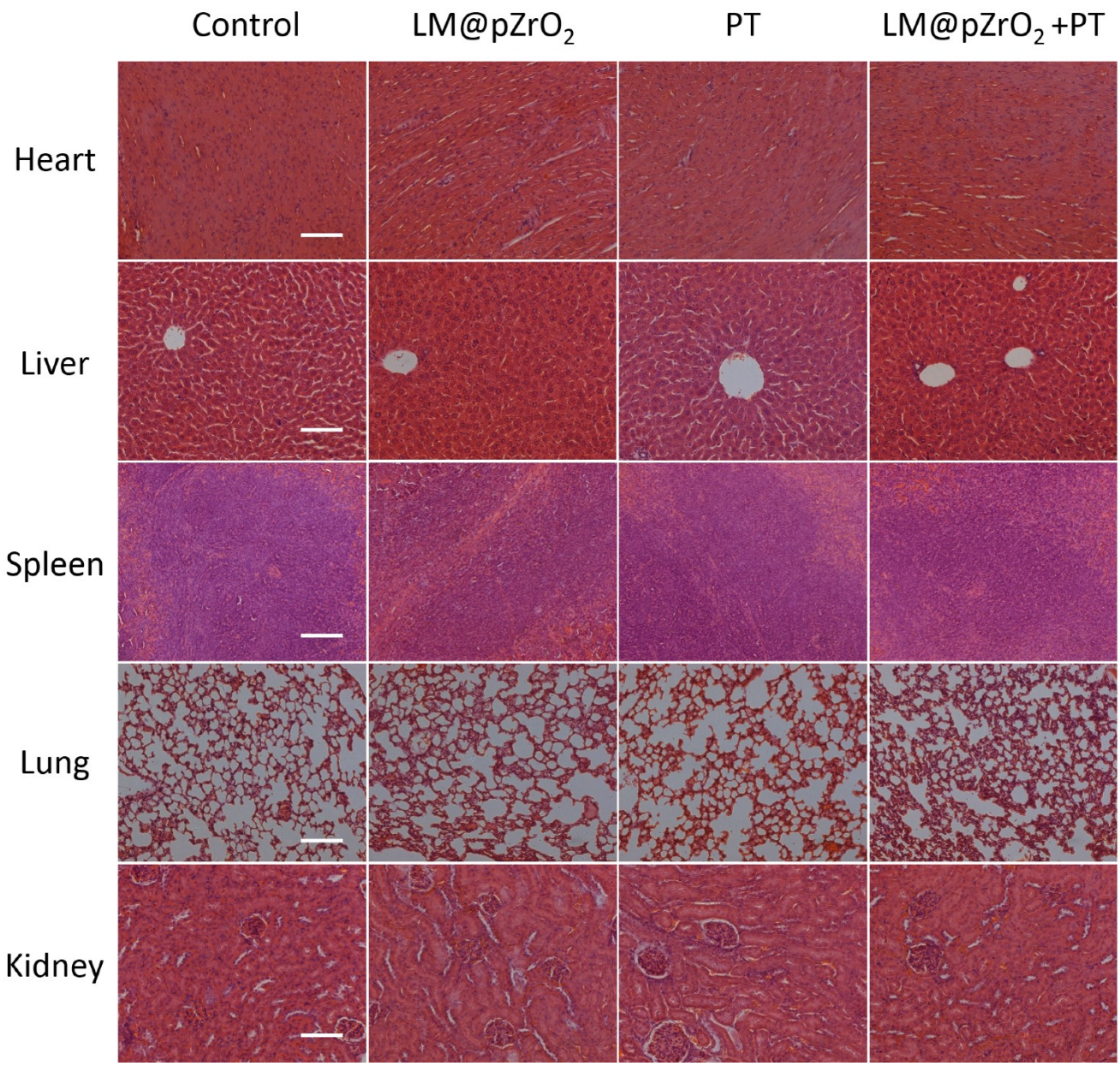

Figure S11. Tissue section (including heart, liver, spleen, lung, kidney) of mice in different groups (control group, $LM@pZrO_2$ group, PT group and $LM@pZrO_2$ + PT group) by paraffin fixation and HE staining. The scale bar was 50 μm.

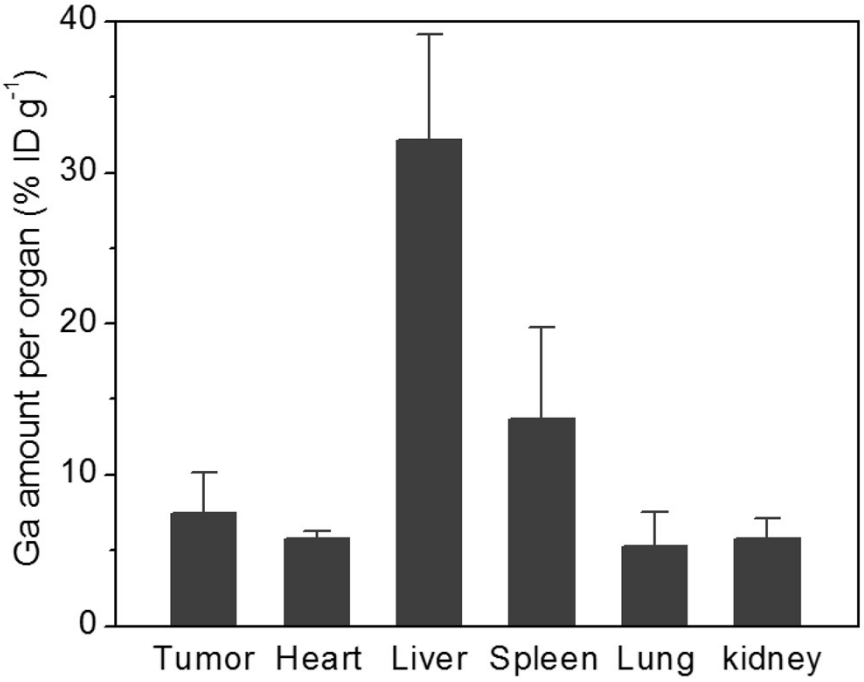

Figure S12. Distribution of Ga in tumors and major tissues from mice at 6 h after injection (the dose of LM@pZrO$_2$ was 50 mg kg^{-1}, n=3).

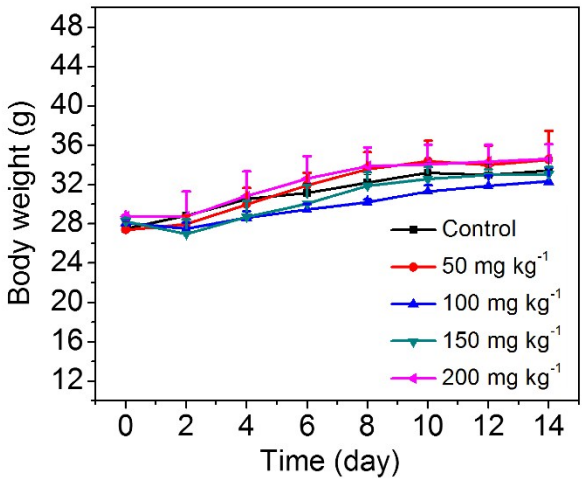

Figure S13. Weight curve of mice within 14 days after mice was injected LM@pZrO$_2$ NPs at different doses (0, 50, 100, 150 and 200 mg kg^{-1}) *via* tail vein.

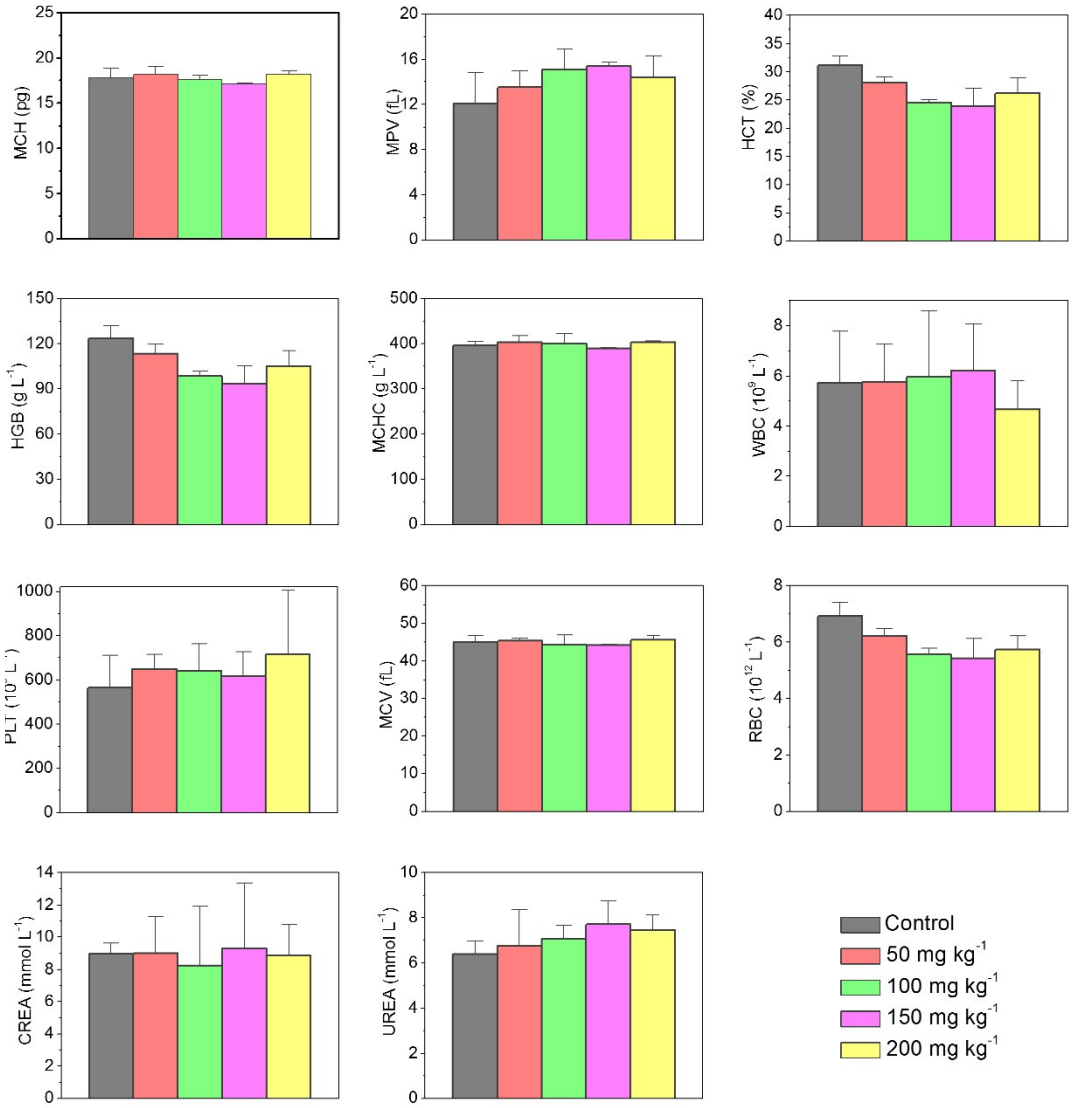

Figure S14. Histogram of blood routine (including MCH, MPV, HCT, HGB, MCHC, WBC, PLT, MCV and RBC) and blood biochemistry (including CREA and UREA) in mice after tail vein injection of LM@pZrO_2 NPs at different doses (0, 50, 100, 150 and 200 mg kg^{-1}) for 14 days.