Supporting Information

Facile synthesis of Fe-*p*-aminophenol nanoparticles for photothermal therapy

Ying Liu,[ab] Sainan Liu,[bc] Chunling Hu,[bc] Yanwei Li*[a] and Maolin Pang*[bc]

[a] School of Materials Science and Engineering, Changchun University of Science and Technology, Changchun, 130022, P. R. China

[b] State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China

[c] University of Science and Technology of China, Hefei 230026, P. R. China

* Corresponding author.
E-mail address: liyanwei@cust.edu.cn; mlpang@ciac.ac.cn.

Experimental Section

Materials: Ferric chloride ($FeCl_3$, AR, Aladdin), P-Aminophenol (PAP, C_8H_7NO, Beijing Chemical Works), Acetonitrile (AR, Aladdin), and Deionized water (Millipore, 18.2 MΩ·cm resistivity at 25 ^oC), all chemicals and chemical reagents were used direct without any further purification.

Characterization: Powder X-ray diffraction (PXRD) measurements were performed on Rigaku MiniFlex 600 at a scanning rate of 10°/min in the 2θ range from 3 to 40°, with graphite monochromatized Cu Kα radiation (λ= 0.15405 nm). Thermogravimetric analysis (TGA) data were recorded with Thermal Analysis Instrument (SDT 2960, TA Instruments, New Castle, DE) with a heating rate of 10 °/min in a nitrogen flow of 100 mL/min. The morphology of the samples was characterized by using a field-emission scanning electron microscope (FE-SEM, S-4800, Hitachi) equipped with an energy-dispersive X-ray (EDX) spectrometer. Transmission electron microscopy (TEM) images were obtained on a FEI Tecnai G2 S-Twin with a field emission gun operating at 200 kV. Fourier transform infrared spectra were measured on a Vertex PerkinElmer 580BIR spectrophotometer (Bruker) with KBr pellet technique. The UV-vis adsorption spectra were measured on a Hitachi U-3100 spectrophotometer. Inductively Coupled Plasma (ICP) was taken on an iCAP 6300 of Thermo scientific. The X-ray photoelectron spectra (XPS) were taken on a VG ESCALAB MK II electron energy spectrometer using Mg KR (1253.6 eV) as the X-ray excitation source. MTT experiments were carried out using a microplate reader (Thermo Multiskan MK3).

Synthesis of Fe-PAP: Fe-PAP was prepared by a simple one-step approach. 3 mg of PAP (0.028 mol) and 0.03 mL of $FeCl_3$ (0.0092 mol, in acetonitrile) were mixed in 1mL of acetonitrile and 1 mL of deionized water (DI water), and then stirred for 12 h at room temperature. The black precipitates were centrifuged and washed with ethanol for three times.

Synthesis of HA modified Fe-PAP: 10 mg of Fe-PAP powder and 4 mg of HA were mixed in 2 mL of DI water, and the mixture was stirred for 3 h, then the precipitates were collected by centrifugation and washed three times with absolute ethanol.

The photothermal conversion efficiency of HA-Fe-PAP: The photothermal conversion efficiency of HA-Fe-PAP was calculated by preciously reported literature.

$$\eta = \frac{hS(T_{max} - T_{sur}) - Q_{Dis}}{I(1 - 10^{-A_{808}})}$$

Where *h* is heat transfer coefficient, *S* is the surface area between container and environment, T_{max} is the maximum temperature of HA-Fe-PAP nanoparticles in the aqueous solution. T_{sur} is the environment temperature. Q_{Dis} is the heat dissipation by the test cell. *I* is the 808 laser power (0.9 w cm^{-2}). A_{808} is the absorbance of HA-Fe-PAP nanoparticles at 808 nm.

$$hS = \frac{m_d C_d}{\tau_s}$$

Where md is the mass (1 g) and Cd is the heat capacity (4.2 J/g) of the aqueous solution, τ_s is the sample system time constant.

Cell toxicity test: The biocompatibility and cytotoxicity of HA-Fe-PAP was evaluated using a standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) test. L929 cells or HeLa cells were placed in a 96-well plate with a density at 6×10^3 cells per well, and incubated in Dulbecco's modified eagle medium (DMEM) containing 10% of fetal bovine serum for 2 h. Then the media were replaced by culture medium containing Fe-PAP with different concentrations (3.75, 7.5, 15, 30, 60, and 120 μg mL^{-1}). For the cytotoxicity test, after incubation for 4 h, all groups were irradiated by an 808 nm laser (0.9 W cm^{-2}) for 5 min, and then incubated for another 24 h. After that, 10 μL of MTT with a concentration of 5 mg mL^{-1} was added and the plates were incubated for 4 h at 37 ^oC. Subsequently, the supernatant was replaced with 150 μL of dimethyl sulfoxide (DMSO) each well. The plate was shaken for 10 min and examined using a microplate reader (Therom Multiskan MK 3) at the wavelength of 490 nm. Cell viability values were calculated by the equation of Cell viability (%) = absorbance of experimental group / the absorbance of control group ×100%.

Preparation of Rhodamine B-conjugated Fe-PAP NPs (Fe-PAP-RhB): 10 mg of EDC, 3 mg of NHS and 5mg of RhB were added into the aqueous of Fe-PAP NPs, the mixture was stirred at room temperature overnight, and then the products were harvested by centrifugation (4000 r/min) and washed several times with ethanol until the supernatant was colorless.

Cellular uptake of the nanoparticles: The HeLa cells were seeded into 6 well at a density 8 x 10^5 per plate, and then incubated with Fe-PAP-RhB for 1, 4, and 6 h, respectively. After that, washed with PBS two times, fixed with 4% paraformaldehyde for 10 min in the dark and then stained with DAPI. The cells uptake process was monitored by intracellular fluorescence microscope.

Flow cytometry measurements: HeLa cells were seeded at a density 2×10^5 per well, and then treated with (a) PBS; (b) NIR; (c) Fe-PAP; (4) NIR＋Fe-PAP, respectively. All cells were incubated for 12 h at 37 ^oC, and then the HeLa cells was washed with PBS for two times, digested with trypsin and handled with PBS (4^o). The cells were stained by Annexin V-fluorescein isothiocyanate (Annexin V-FITC) and propidium iodide (PI), and then cell apoptosis rate was measured by a FACS Calibur flow cytometer (BD Biosciences).

In vivo antitumor efficacy of Fe-PAP: Female Balb/C mice were purchased from the Center of Experimental Animals, Jilin University (Changchun, China), and the animal experiments agreed with the criterions of The National Regulation of China for Care and Use of Laboratory Animals. H22 cancer cells (murine liver cancer) were injected in the left axilla of Balb/C mice to establish a mice model. After the tumor volume reached 80-120 mm^3, the mice were randomly divided into 4 groups (n=5, per group), and treated with Phosphate Buffered Saline (PBS), PBS + NIR, Fe-PAP and Fe-PAP + NIR, respectively. For PBS + NIR and Fe-PAP + NIR groups, the tumor was irradiated with an 808 nm laser for 5 min (0.9 w cm^{-2}) on day 1 and 7, respectively. Body weight and tumor size were monitored every two days for two weeks. The tumor volume was calculated by V = 4/3 x Length x $width^2/8$. The relative tumor volume was calculated as V/V_0, where V_0 was the tumor volume before the treatment. Finally, the major organs of mice, such as liver, spleen, heart, lung, and kidney, were removed and fixed in 4% paraformaldehyde solution for histological examination to further investigate the biocompatibility of Fe-PAP.

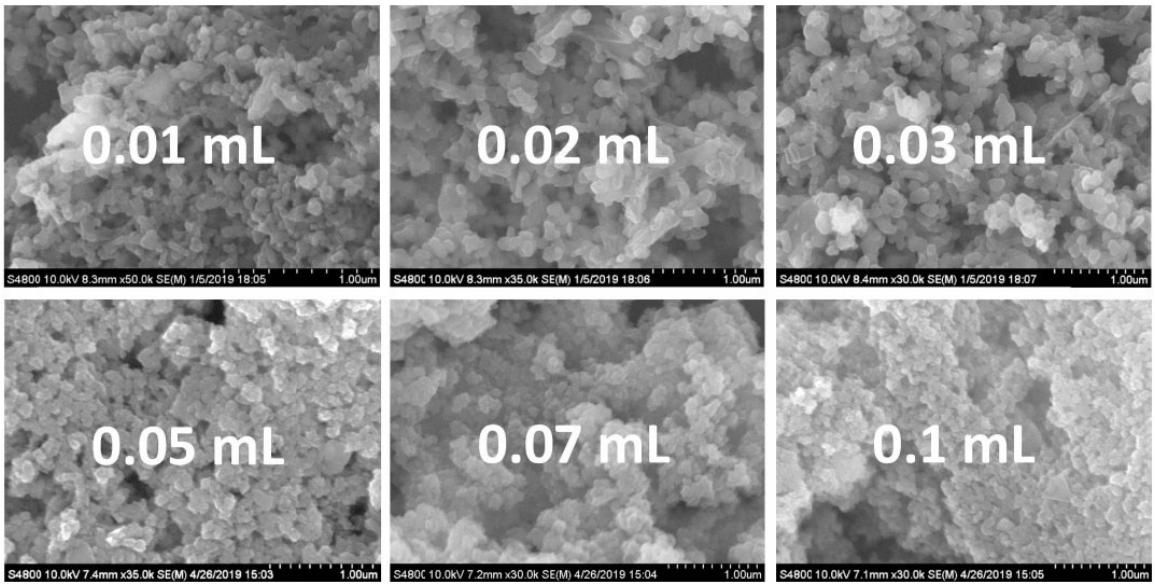

Fig. S1 SEM images of Fe-PAP prepared in the presence of different amounts of $FeCl_3$.

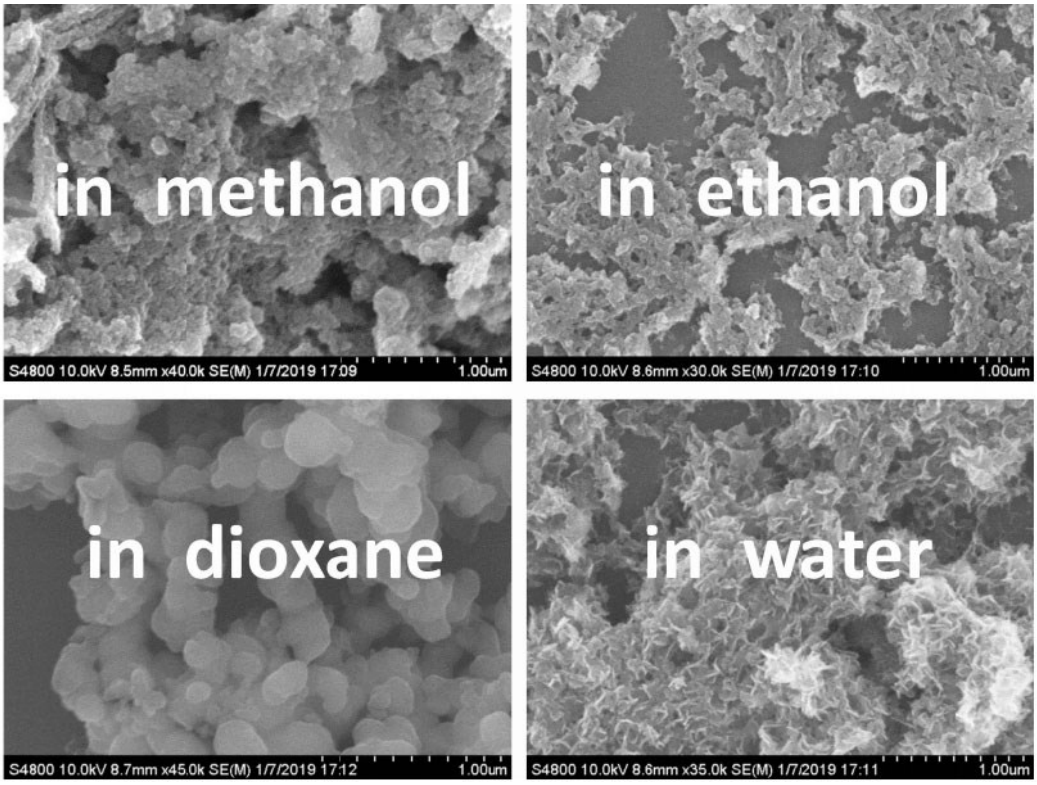

Fig. S2 SEM images of Fe-PAP synthesized in different solvents.

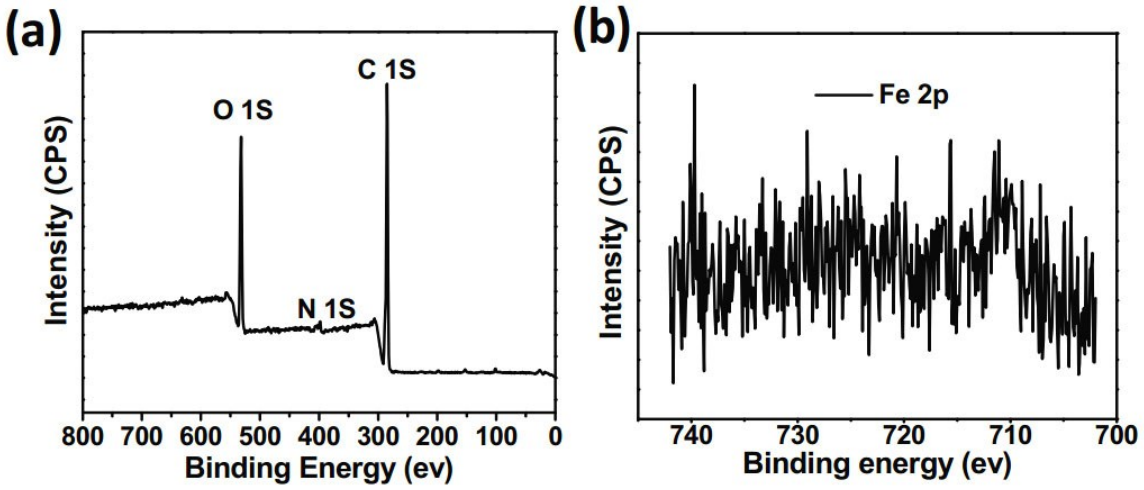

Fig. S3 XPS spectra of Fe-PAP.

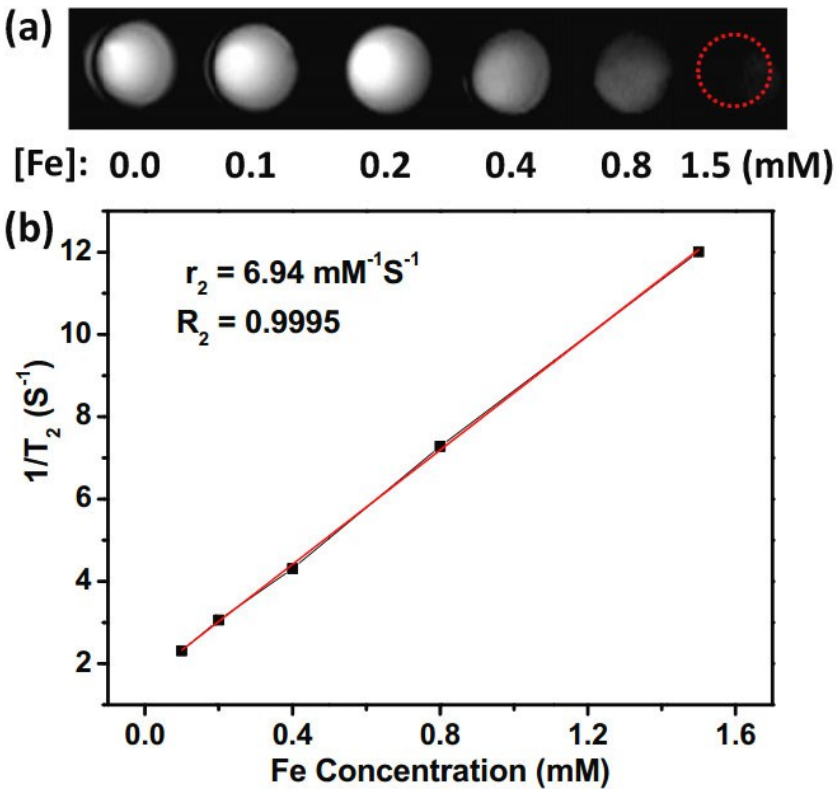

Fig. S4 (a) T_2-weighted MR images of HA-Fe-PAP dispersed in water with different concentrations, and (b) relaxation rate R_2 ($1/T_2$) versus concentrations of HA-Fe-PAP.

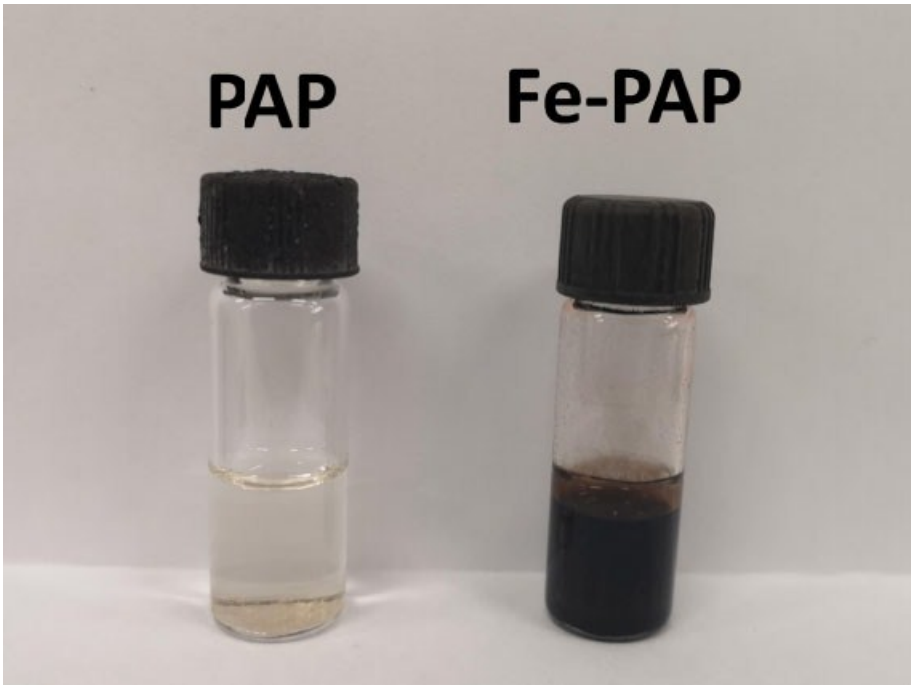

Fig. S5 Photographs of the precursor and the as-synthesized product.

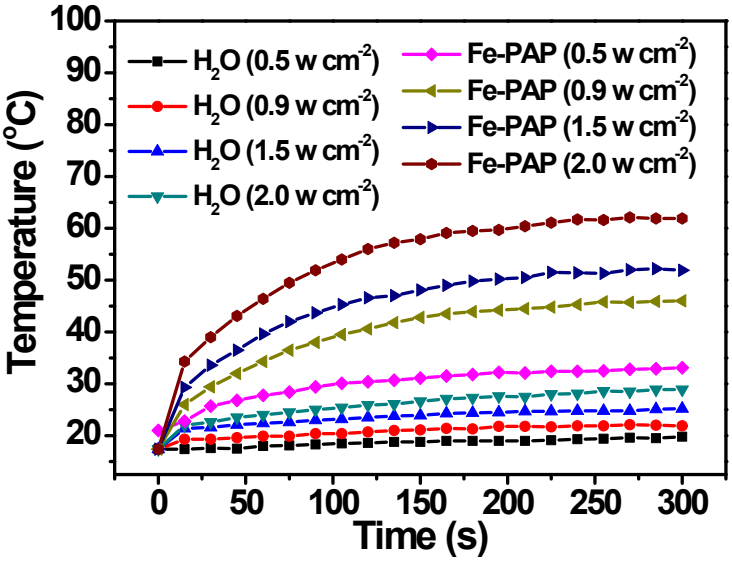

Fig. S6 The temperature variation curves of pure water and HA-Fe-PAP (120 μg mL^{-1}) irradiated by 808 nm laser with different power densities.

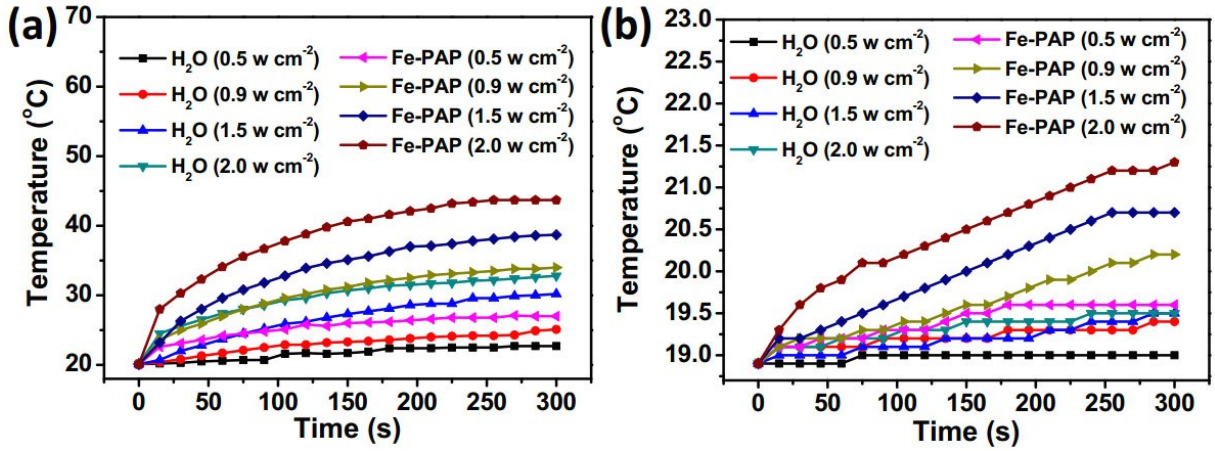

Fig. S7 The temperature variation curves of pure water and HA-Fe-PAP (120 μg mL^{-1}) under (a) 1064 nm and (b) 980 nm laser irradiation, respectively.

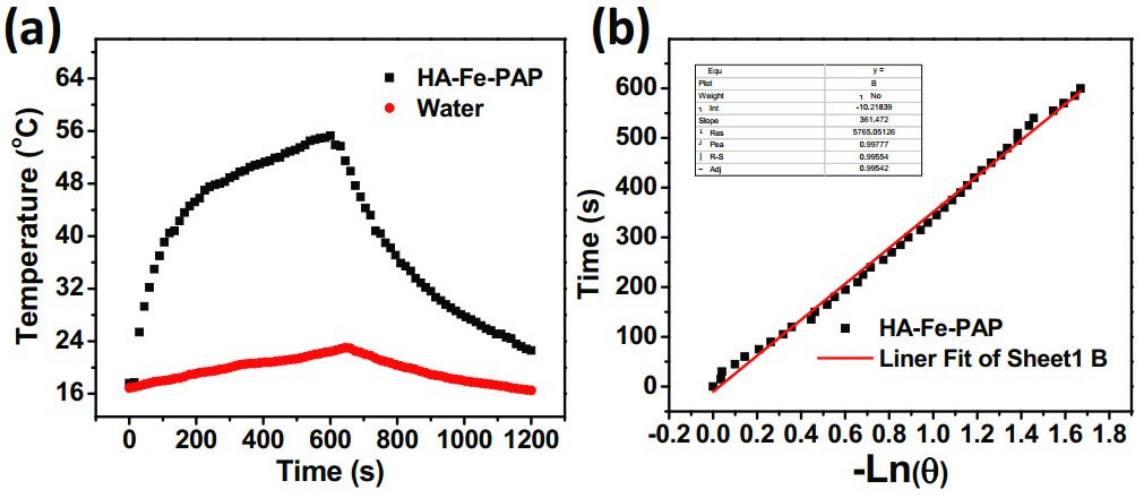

Fig. S8 (a) Photothermal effect of Fe-PAP aqueous solution (120 μg mL^{-1}) irradiated with an 808 nm laser for 10 min (0.9 W cm^{-2}). (b) Linear fit of time/-ln(θ) obtained during the cooling process.

Table S1. Photothermal conversion efficiency of HA-Fe-PAP.

A_{808}	T_{max}-T_{surr}	τ_s	η
0.768	40.3	361.472	36%

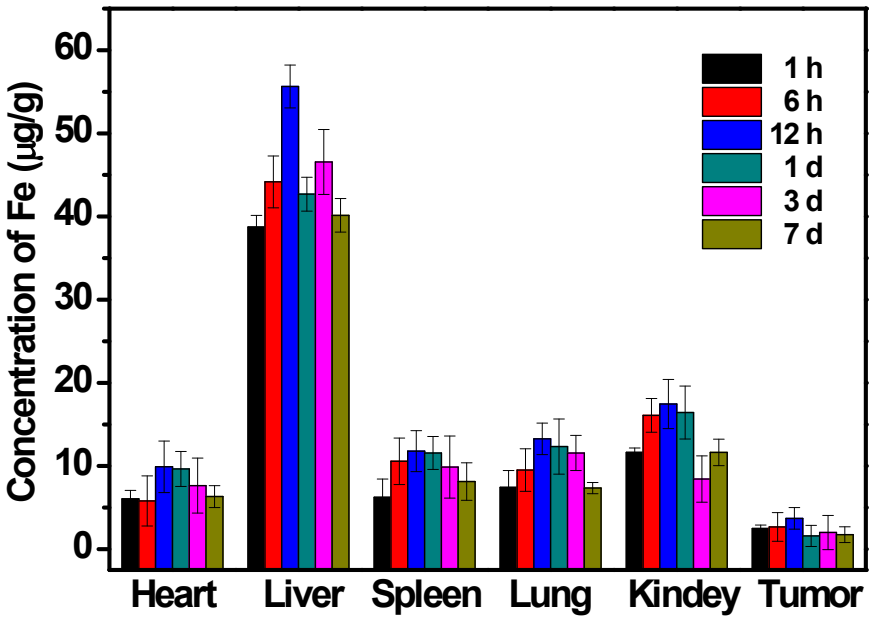

Fig. S9 The in vivo biodistribution of HA-Fe-PAP nanoparticles after tail vein injection.