Supporting Information

Facile Preparation of Ion-Doped Poly(*p*-phenylenediamine) Nanoparticles for Photothermal Therapy

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Chemicals and Materials. Ferric chloride ($FeCl_3$, AR, Aladdin), *p*-Phenylenediamine ($C_6H_8N_2$, AR, 97.0%, Aladdin), Methanol and Ethanol (AR, Beijing Chemical Works), Acetonitrile (CH_3CN, 99.8%, Vetec), and Ammonium persulphate ($(NH_4)_2S_2O_8$, AR, Beijing Chemical Works). All of the chemicals and solvents were used as received without further purification.

Synthesis of Fe-ppd. Fe-ppd was synthesized according to the reported procedure with slight modification.[S1] After ppd (2 mg, 0.01849 mmol) was dissolved in 2 mL of acetonitrile in a 4 mL capped vial, $FeCl_3$ (0.03 mL, 0.308 M, in 0.03 mL of acetonitrile) was added. Then the mixture was stirred at room temperature for 18 hours. The products were harvested by centrifugation at 10,000 rpm for 15 min, and washed with ethanol for three times, finally dried at 70 °C for 12 hours.

Synthesis of NH_2-PEG-2000-NH_2 modified Fe-ppd. In a typical procedure, solid Fe-ppd (10 mg) and NH_2-PEG-2000-NH_2 (4 mg) were mixed in 3 mL of deionized water under magnetic stirring. After 6 h, the precipitates were collected by centrifugation and washed with anhydrous ethanol several times. All the experimental were carried out at room temperature.

Synthesis of APS-ppd. 500 μL of 0.1 M ppd aqueous solution was diluted with water to 5.0 mL, followed by the introduction of 100 L of 0.1 M $(NH_4)_2S_2O_8$ (APS) aqueous solution. Then shaking was stopped after 0.5 min, and the reaction solution was kept at 25 °C for 24 h. The dark purple product was collected by filtration, washed two times with distilled water and then dried in vacuum. The resulting precipitate was then dispersed in water and stored for characterization and further use.[S2]

Photothermal heating experiments on NH_2-PEG-2000-NH_2 modified Fe-ppd. NH_2-PEG-2000-NH_2 modified Fe-ppd aqueous solutions with different concentrations (0-120 μg/mL) were suspended in different wells of a 96 well plate, and irradiated by 808 nm CW laser with a power density of 1.25 W/cm^2 for different times. The temperatures were carefully measured by a digital thermometer with a thermocouple probe. To a certain Fe-ppd concentration (60 μg/mL), the heating and cooling (ON-OFF) cycle for photothermal stability was evaluated for more than ten times upon CW laser irradiation (808 nm, 1.57 W/cm^2). In each heating-cooling cycle, CW laser irradiation lasted for 5 min followed by a 20 min cooling period until it reached room temperature again. The in vivo photothermal effect was conducted by intratumoral injection of NH_2-PEG-2000-NH_2 modified Fe-ppd (300 μg/mL) into the tumor on a Balb/C mouse with a power density of 1.57 W/cm^2, and the temperatures were recorded every 15 s by a photothermal camera.

Photothermal efficiency of NH_2-PEG-2000-NH_2 modified Fe-ppd. The photothermal efficiency was determined based on the protocol reported before.[S3] First, The Fe-ppd aqueous solution was irradiated by CW laser for 10 min (808 nm, 1.57 W/cm^2). Then the heated aqueous dispersion cooled down naturally and the temperatures during the cooling process were also carefully monitored every 15 s by a thermometer with a thermocouple probe. The photothermal conversion efficiency (η) was calculated by the following equation:

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

where h is the heat transfer coefficient, S is the surface area of the container, T_{max} is the equilibrium temperature after 10 min irradiation, Q_{Dis} expresses the heat dissipation by the test cell, I is 808 nm CW laser power (1.57 W/cm^2), and A_{808} is the absorbance of the Fe-ppd aqueous solution at 808 nm. The value of hS is determined according to the following equation:

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

Where m_d is the mass (1 g) and C_d is the heat capacity (4.2 J/g) of the aqueous solvent, τ_s is the sample system time constant, and θ is defined as the ratio of ΔT and ΔT_{max}.

$$\mathrm{t} = -\tau_s(\ln\theta)$$

$$\triangle T = 26.5\,^{\circ}\mathrm{C},\ A_{808} = 1.318,\ I = 1.58\ \mathrm{W/cm^2}$$

$$\eta = \frac{hS\Delta T_{max} - Q_{Dis}}{I(1-10^{-A_{808}})} = 39.27\%$$

The photothermal efficiency of APS-ppd was also calculated by the same method. The photothermal efficiency and related data of both Fe-ppd and APS-ppd were shown in Table S1 and S2.

In vitro cytotoxicity of NH_2-PEG-2000-NH_2 modified Fe-ppd. The biocompatibility of NH_2-PEG-2000-NH_2 modified Fe-ppd was evaluated using a standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) test. The L929 cells were seeded in a 96-well plate (8000 cells per well) and incubated in a humidified atmosphere of 5% CO_2 overnight to ensure that the cells had attached to the wells. After that, NH_2-PEG-2000-NH_2 modified Fe-ppd with different concentrations (0.9375, 1.875, 3.75, 7.5, 15, 30, 60 and 120 μg/mL) were added into each well, and incubated for another 24 h. MTT solution (10 μL, 5 mg/mL) was added into each well, and incubated for 4 h; then 150 μL of DMSO were added after removing the original culture medium. The final fraction surviving of HeLa cells was measured by a microplate reader at the wavelength of 630 nm. In vitro cytotoxicity of NH_2-PEG-2000-NH_2 modified Fe-ppd was assayed against HeLa cells under similar experimental conditions as described above. For the photothermal heating experiments, after incubation for 4 h, the NH_2-PEG-2000-NH_2 modified Fe-ppd were removed by rinsing three times with PBS, fresh culture medium was then added into the wells. The cells were exposed to NIR light (1.11 W/cm^2) for 5 min to conduct the photothermal treatment, and then continued incubated at 37 °C in 5% CO_2 for 24 h. While for the control group, it was incubated with cells for 24 h without additional treatment. Finally, the viability of the HeLa cells was evaluated using a microplate reader at 630 nm.

In vivo antitumor efficacy of NH_2-PEG-2000-NH_2 modified Fe-ppd. Female Balb/C mice (about 18 g) 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. The tumors were established by subcutaneous injection of H22 cells (murine hepatocarcinoma cell lines) in the left axilla of the mice. The tumor bearing mice were randomly divided into four groups (n=8, each group) after the size of the tumors reached 80-120 mm^3, and treated with PBS (control), PBS + NIR, Fe-ppd-PEG and Fe-ppd-PEG + NIR by intratumoral injection, respectively. The body weight and tumor volume of each mouse were monitored every two days, and after 14 days treatment, the tumors were dissected and weighed to evaluate the therapeutic efficacy. In a typical calculation, the tumor volume was calculated by $V = 4/3 \times \text{Length} \times \text{width}^2/8$. The relative tumor volume was calculated as V/V_0, where V_0 was the tumor volume before the treatment. Tumor growth inhibition rate was determined as $(C - T)/C \times 100\%$, in which C was the average tumor weight of the control group, while T is the average tumor weight of each treated group. 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 in order to further investigate the biocompatibility of Fe-ppd.

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).

References:

S1. Y. S. Shi, X. R. Deng, S. X. Bao, B. Liu, B. Liu, P. A. Ma, Z. Y. Cheng, M. L. Pang and J. Lin, *Chem. Asian J.*, 2017, **12**, 2183.

S2. S. Yang, S. Huang, D. Liu and F. Liao, *Synth. Met.*, 2012, **162**, 2228.

S3. D. K. Roper, W. Ahn and M. Hoepfner, *J. Phys. Chem. C*, 2007, **111**, 3636.

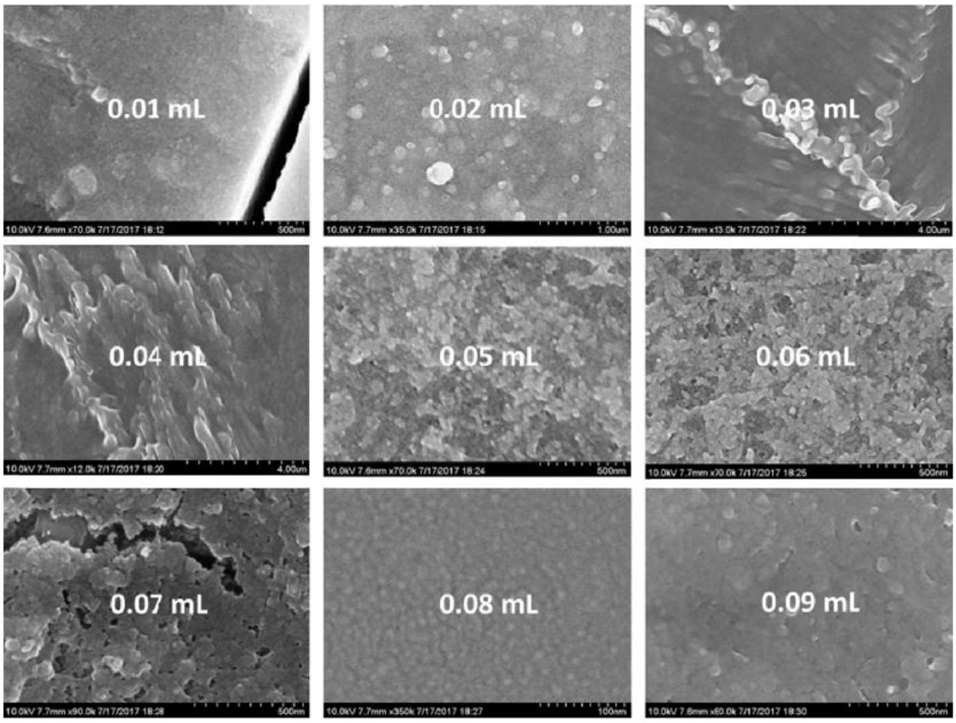

Fig. S1 SEM images of Fe-ppd prepared in the presence of different amounts of $FeCl_3$.

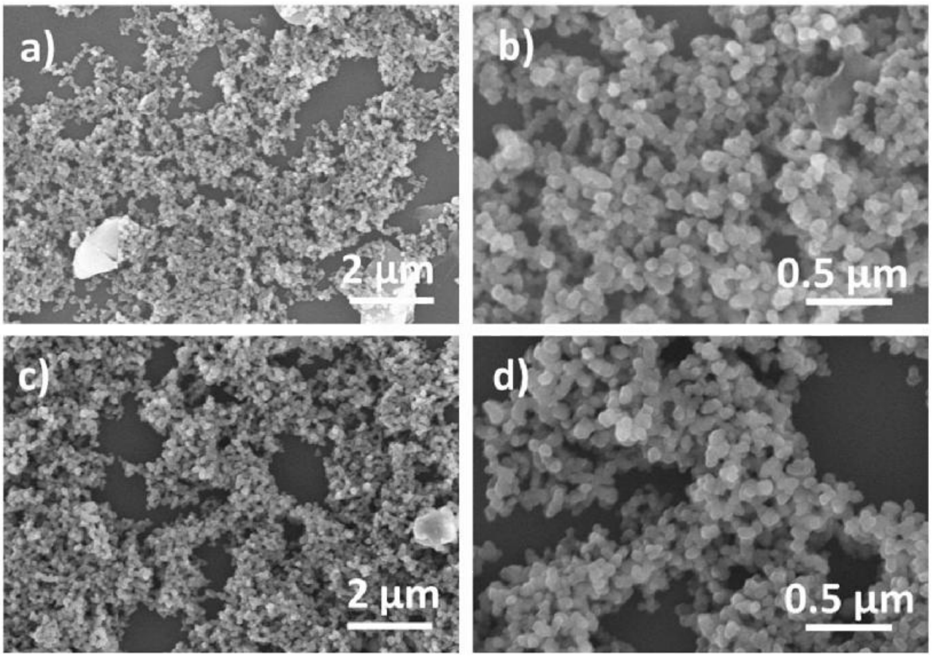

Fig. S2 SEM images of Fe-ppd synthesized in (a), (b) co-solvents of methanol and acetonitrile, and (c), (d) acetonitrile.

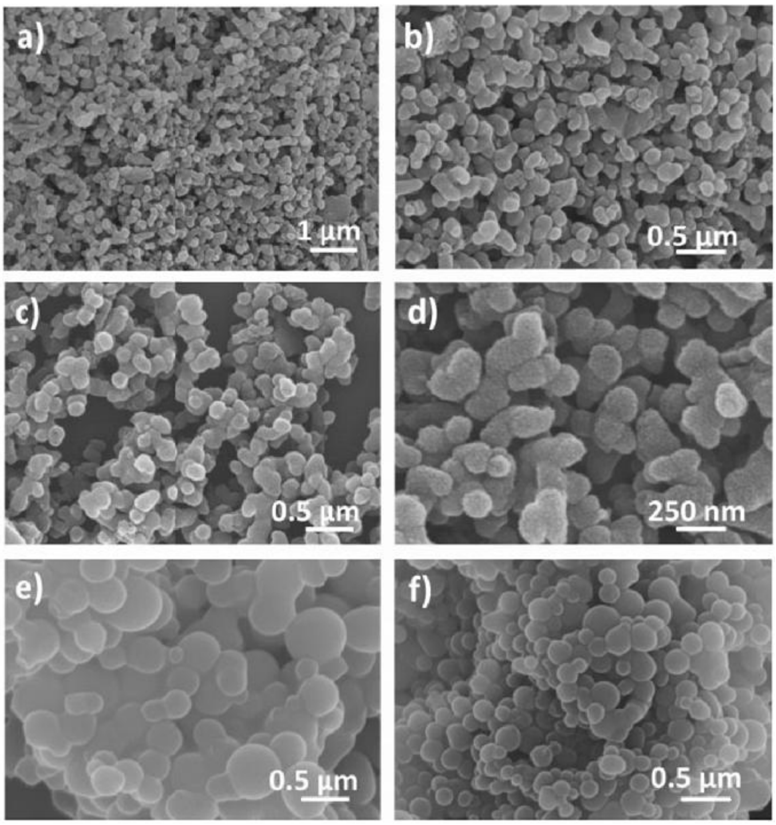

Fig. S3 SEM images of (a-d) Fe-ppd, (e) and (f) Fe-ppd modified with NH_2-PEG-2000- NH_2.

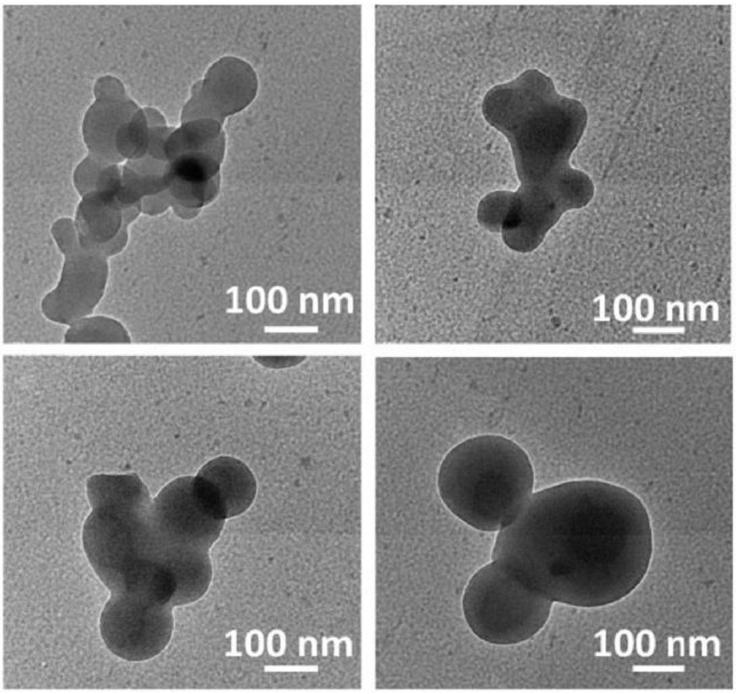

Fig. S4 TEM images of Fe-ppd.

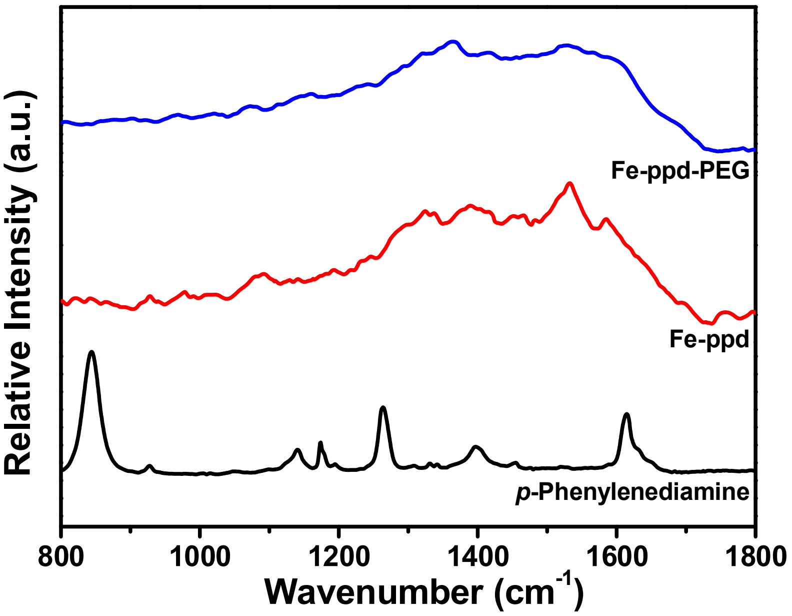

Fig. S5 Raman spectra of ppd, Fe-ppd, and Fe-ppd-PEG.

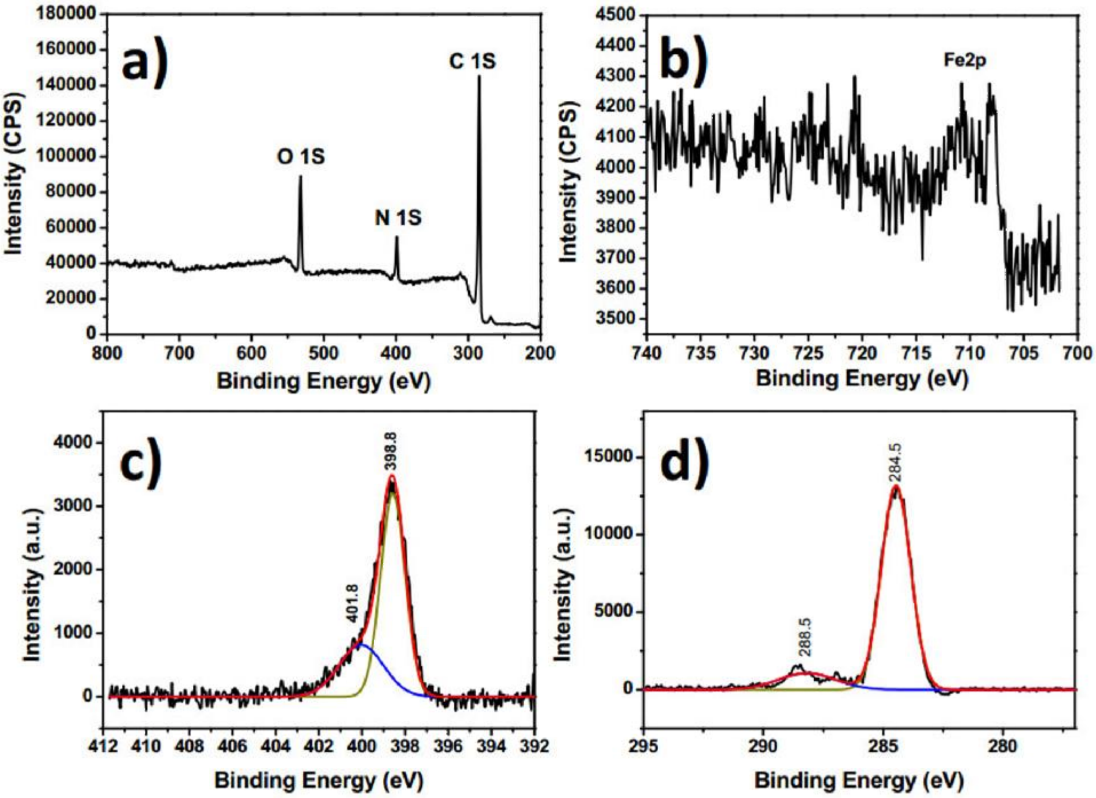

Fig. S6 XPS spectra of Fe-ppd.

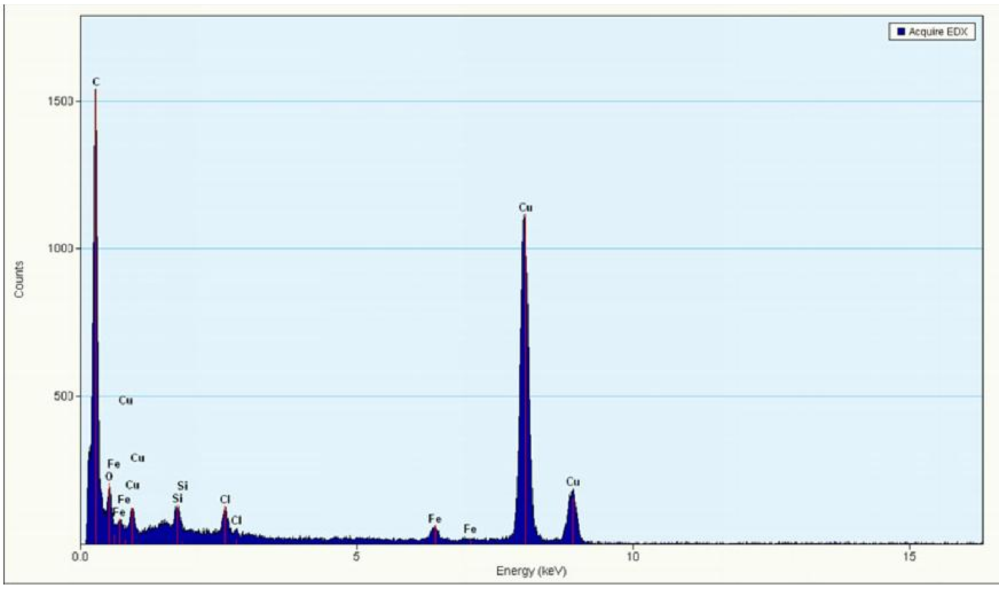

Fig. S7 EDS of Fe-ppd.

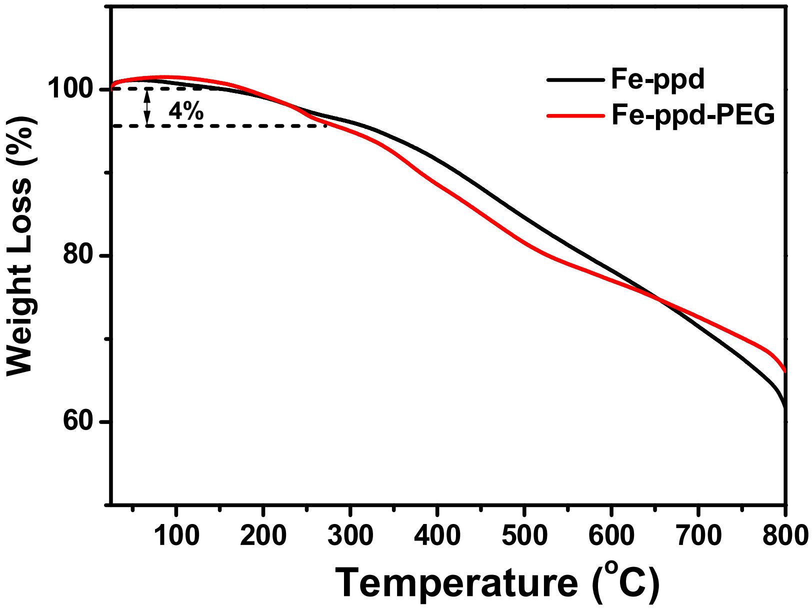

Fig. S8 TGA curves of Fe-ppd and Fe-ppd-PEG.

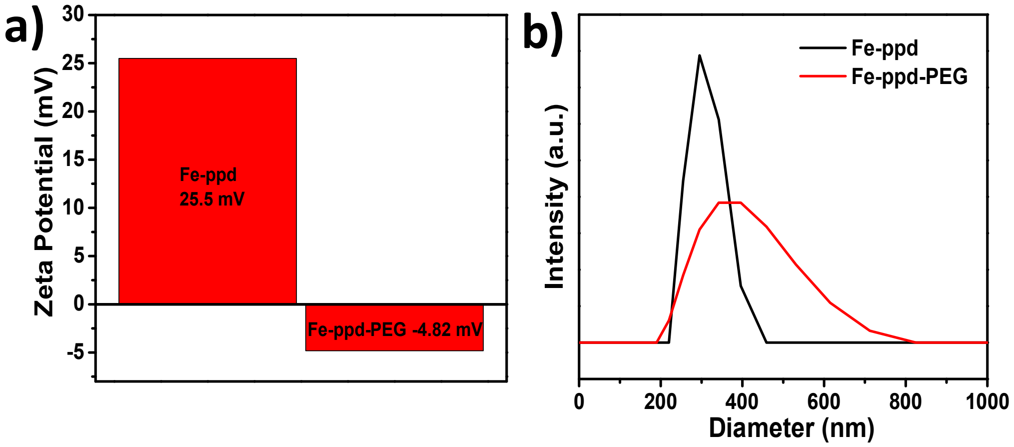

Fig. S9 The zeta potential and DLS measurement results for Fe-ppd and Fe-ppd-PEG.

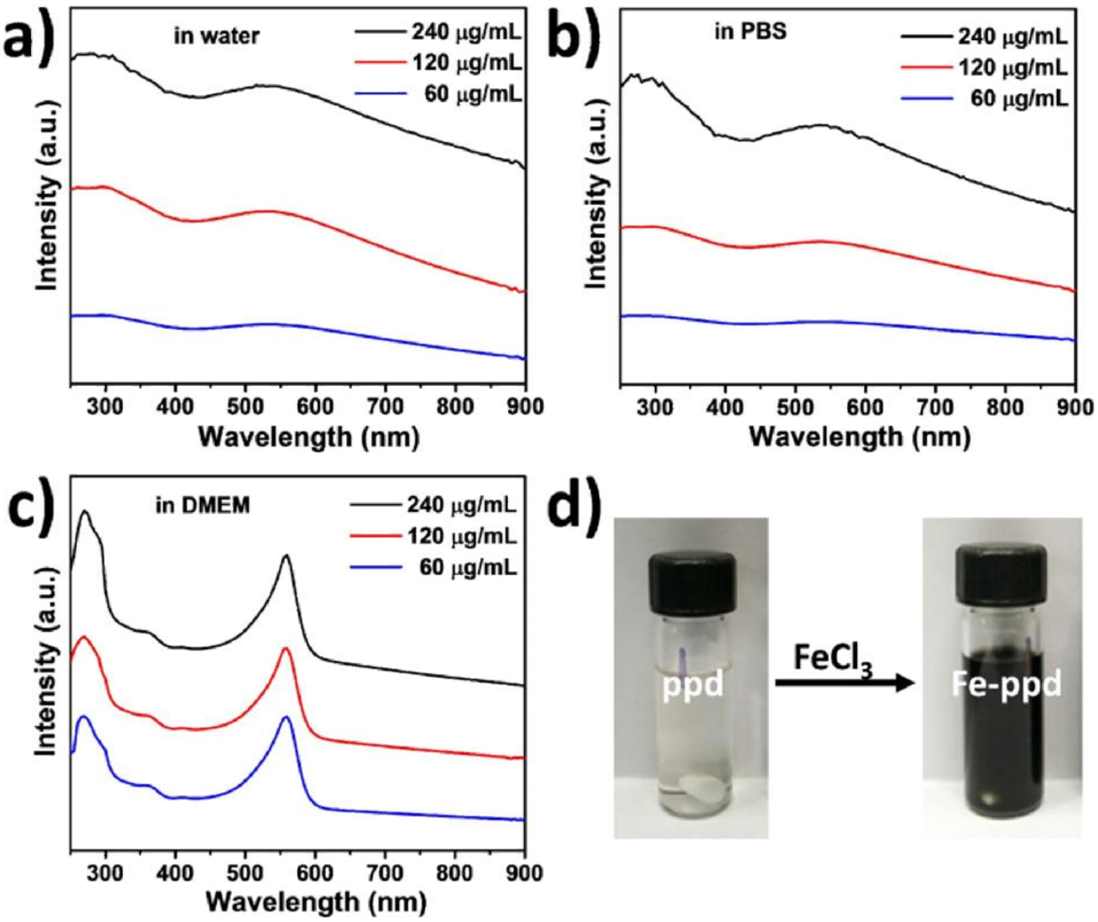

Fig. S10 UV-vis spectra of Fe-ppd dispersed in (a) DMEM, (b) distilled water, and (c) PBS buffer solution, respectively.

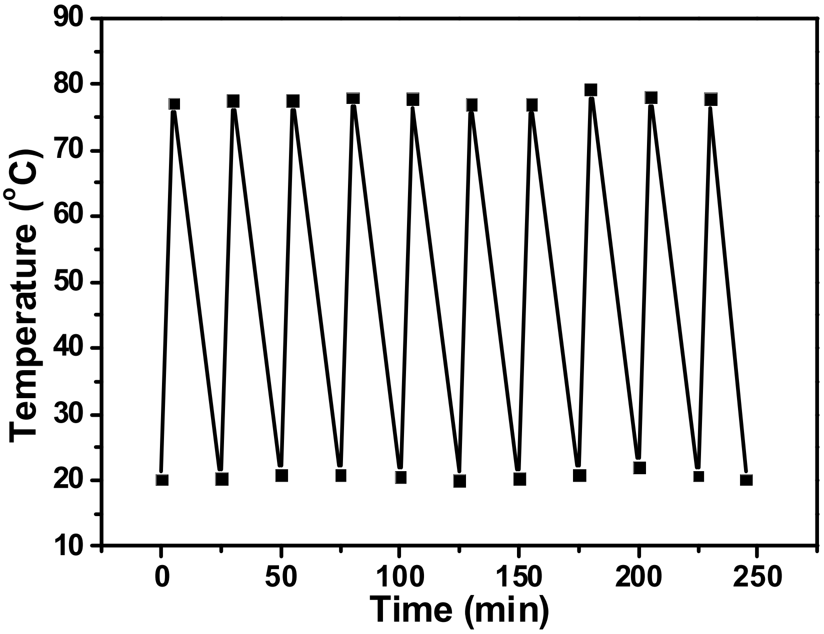

Fig. S11 Photostability test for Fe-ppd in 0.4 mL of DMEM (60 µg/mL, 1.35 W/cm^2).

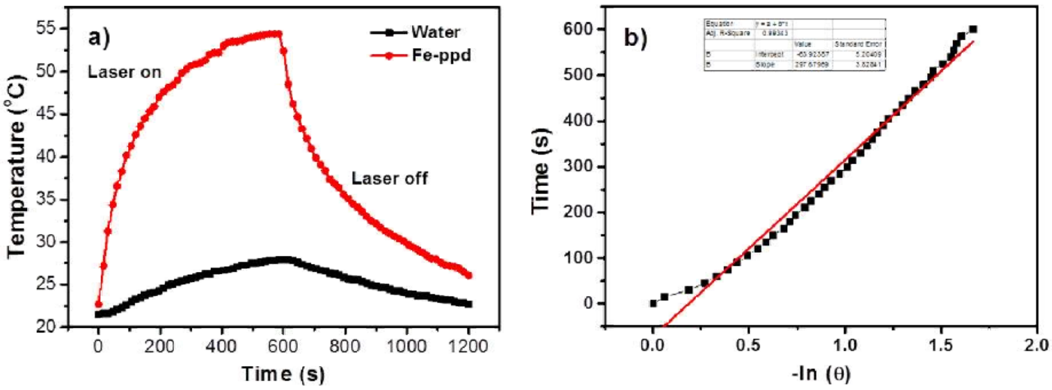

Fig. S12 (a) Photothermal effect of Fe-ppd aqueous solution (120 µg/mL) irradiated with 808 nm CW laser (1.57 W/cm^2). (b) Linear fit of time/-ln(θ) obtained during the cooling process.

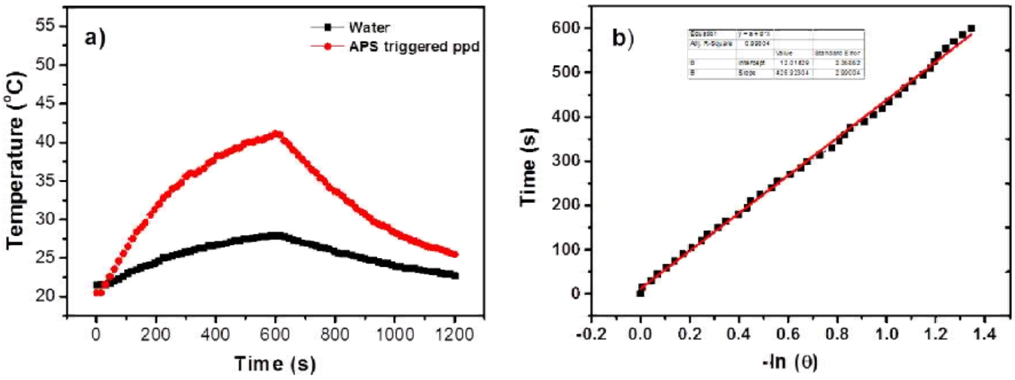

Fig. S13 (a) Photothermal effect of APS-ppd aqueous solution (120 μg/mL) irradiated with 808 nm CW laser (1.57 W/cm^2). (b) Linear fit of time/-ln(θ) obtained during the cooling process.

Table S1. Photothermal conversion efficiency for NH_2-PEG-2000-NH_2 modified Fe-ppd. Absorbance at irradiation wavelength (A808 nm), mass of solution (m sol), increasing temperature after CW laser irradiation (ΔT), time system constant (τ_s), thermal conductance (hS) and photothermal conversion efficiency (Efficiency)

Abs 808nm	**m sol (g)**	**$\triangle T$ (K)**	**T_s (s)**	**hS (WK^{-1})**	**Efficiency (%)**
1.318	1	26.5	297.68	0.0141	39.27

Table S2. Photothermal conversion efficiency for APS-ppd.

Abs 808nm	**m sol (g)**	**$\triangle T$ (K)**	**T_s (s)**	**hS (WK^{-1})**	**Efficiency (%)**
0.992	1	20.6	426.92	0.0098	22.56

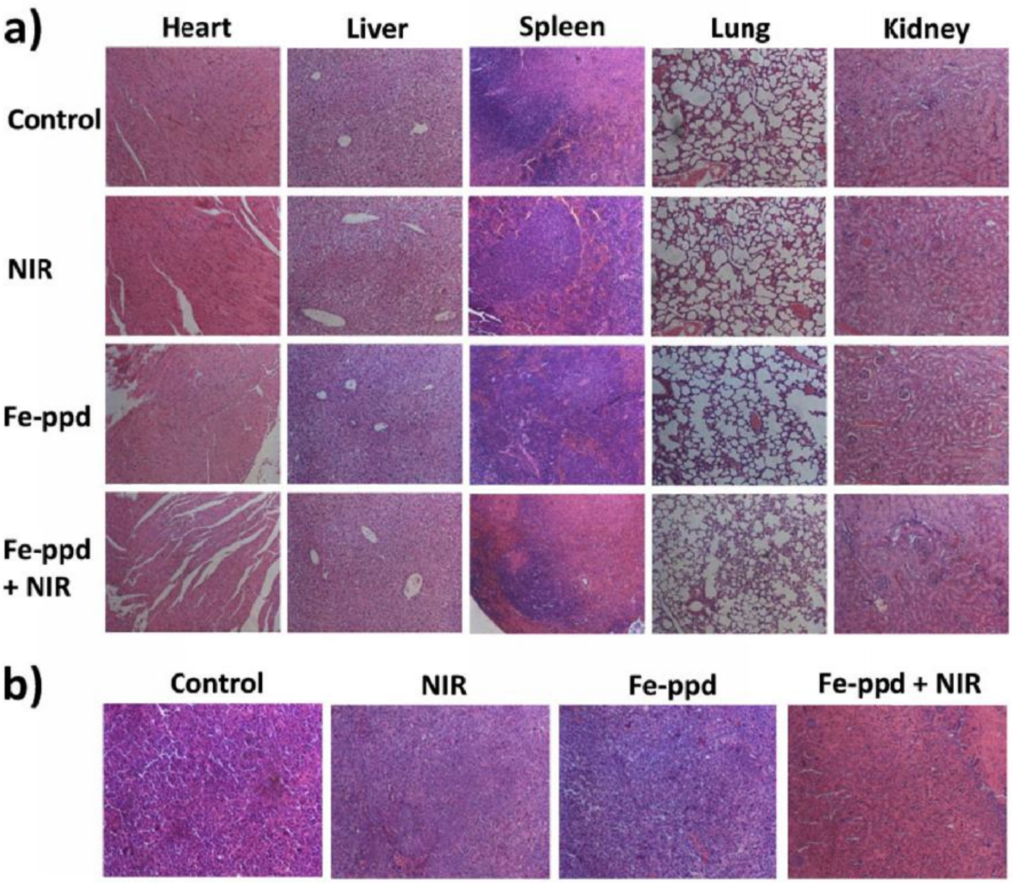

Fig. S14 Hematoxylin and eosin (H&E) stained images of (a) major organs and (b) tumor slices of mice for control, NIR, Fe-ppd-PEG, and Fe-ppd-PEG + NIR groups, respectively.