Electronic Supplementary Information (ESI) for:

Iron Phosphide Nanoparticles as a pH-Responsive T_1 Contrast Agent for Magnetic Resonance Tumor Imaging

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Experimental Section

Materials

Tris(acetylacetonato)iron(III) ($Fe(acac)_3$), Oleylamine (OAm), and Tri-n-octylphosphine (TOP) were purchased from Sigma-Aldrich. DSPE-PEG2000 was purchased from A.V.T. Pharmaceutical Co., Ltd. (Shanghai).

Characterisation

Transmission electron microscopy (TEM) image and energy-dispersive X-ray spectroscopy (EDS) were collected on a Hitachi HT7700 microscope at an accelerating voltage of 100 kV. High-resolution TEM (HRTEM) image were obtained by a Tecnai G2 F20 microscope at an accelerating voltage of 200 kV. X-ray powder diffraction (XRD) pattern was measured on a X'pert^3 Powder diffractometer. Fourier transform infrared (FTIR) spectrum was collected on a Nicolet 6700 spectrometer. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer nano ZS instrument. The Fe concentration of FeP nanoparticles solutions was determined by a XSeries 2 inductively coupled plasma mass spectrometry (ICP-MS).

Synthesis of FeP nanoparticles

To synthesize FeP nanoparticles, 2 g of $Fe(acac)_3$ and 20 mL of OAm were first mixed, and then 9 mL of TOP was dropwise added to the mixture under stirring. Under a nitrogen atmosphere, the mixture was heated to 120 °C for 30 min. Subsequently, the mixture was further heated to 350 °C for another 2 h. After the reaction solution was cooled down to room temperature, 90 mL of isopropanol was added, and the product was collected by centrifugation and then washed with ethanol for several times. The as-synthesized FeP nanoparticles were then modified with PEG. Briefly, ~8 mg FeP nanoparticles were dispersed in 1 mL of chloroform, and ~8 mg DSPE-PEG2000 was dispersed in another 0.5 mL chloroform. Subsequently, two solutions were sufficiently mixed by sonication. The mixture was placed in a fume hood until the solution was completely volatilized. The precipitate product was collected and re-dispersed in water.

In vitro MRI

In vitro MRI measurements were performed on a 0.5 T NMR120-Analyst NMR system (Niumag Corporation, Shanghai, China). The longitudinal relaxation times (T_1) were collected by an inversion recovery (IR) sequence. The longitudinal relaxivity (r_1) was determined from the slope of the plot of $1/T_1$ (s^{-1}) against [Fe] concentrations (mM). T_1-weighted phantom images were

acquired using a 2D multi-slice spin-echo (MSE) sequence with the following parameters: TR/TE = 100/2 ms, thickness = 1 mm, 512 × 512 matrices, slices = 1, and NS = 4. FeP nanoparticles were dispersed in phosphate buffered saline (PBS) with different pH values (pH 5.0, pH 6.0, and pH 7.4), and relaxation times or phantom images were collected at different time points. To investigate the pH-responsive imaging ability of FeP nanoparticles in cells, 1×10^7 MCF-7 cells were first incubated with FeP nanoparticles (25 μg mL^{-1}) at 37 °C for 2 h, 8 h, and 24 h, respectively. Subsequently, the cells were harvested and washed with phosphate buffered saline three times to remove the free FeP nanoparticles. Finally, the cells were collected at the button of small tubes by centrifugation for T_1-weighted phantom imaging.

***In vivo* MRI**

Animal experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and were approved by Institutional Animal Care and Use Committee of Fujian Medical University. To induce a solid tumor, male BALB/c mice (~20 g) were subcutaneously injected MCF-7 cells (~5×10^6 cells in 100 μL PBS) in right thigh areas. The mice were used when the tumor grew to ~2 cm. T_1-weighted images of the mice before the injection were first collected as controls. The mice were then intravenously injected with 100 μL FeP nanoparticles with a dosage of 6 mg Fe/kg. Same slices were further collected at 2 h, 8 h, and 24 h after the injection. All the images were obtained on a GE Discovery MR750 MRI scanner using a FSE sequence under the following parameters: TR/TE = 663/10.3 ms, FOV = 8 × 8 cm, 320 × 224 matrices, thickness = 1 mm. To quantify the contrast enhancement, the signal-to-noise ratio (SNR) was measured by finely analyzing regions of interest (ROIs) in tumor areas, and the contrast enhancement was defined as the increase of SNR after the injection, $\triangle SNR = (SNR_{post} - SNR_{pre})/SNR_{pre}$. To investigate the biodistribution of FeP nanoparticles, the mice were first injected with FeP nanoparticles (60 mg Fe/kg). After 10 h, the mice were then sacrificed, and heart, liver, spleen, lung, kidney, and tumor were collected for analyses. The tissue samples were treated with HNO_3-H_2O_2 digestion. Finally, Fe concentrations in these samples was measured by ICP-MS. To determine the basic Fe concentration in these organs, five mice were investigated using the similar procedure except without the injection of FeP nanoparticles.

Cytotoxicity assay

The cytotoxicity of FeP nanoparticles was evaluated by 3-(4, 5-dimethylthiazol-2-y1)-2, 5-

diphenyltetrazolium bromide (MTT) assay. MCF-7 or L02 cells were first seeded into a 96-well plate with a density of 1×10^4 cells/well and incubated in 5% CO_2 atmosphere at 37 °C for 24 h. The cells were then incubated with FeP nanoparticles of different concentrations for another 24 h. After that, the culture medium was discarded, and each well was added with 100 μL of new culture medium containing 0.5 mg mL^{-1} MTT. The plate was incubated for another 4 h at 37 °C. Finally, the medium was discarded and each well was added 200 μL DMSO. The OD_{490} value (Abs.) of each well was measured by an SH-1000 Lab microplate reader immediately. The cell viability was calculated by OD_{490} value of experimental group subtracting that of blank group.

***In vivo* biocompatibility study**

The healthy male BALB/c mice were intravenously injected with 100 μL FeP nanoparticles (dosage of 6 mg Fe/kg). 14 days after the injection, the blood was collected from the mice for blood biochemistry and hematology analyses. Reference intervals of blood biochemistry and hematology for healthy BALB/c mouse were obtained from Charles River Laboratories (https://www.criver.com/sites/default/files/resources/BALBcMouseClinicalPathologyData.pdf).

The major organs including heart, liver, spleen, lung, and kidney were harvested for histology analyses. The tissue sections with thickness of 4 μm were prepared and stained with hematoxylin and eosin (H&E) following the standard protocol.

Supporting Figures

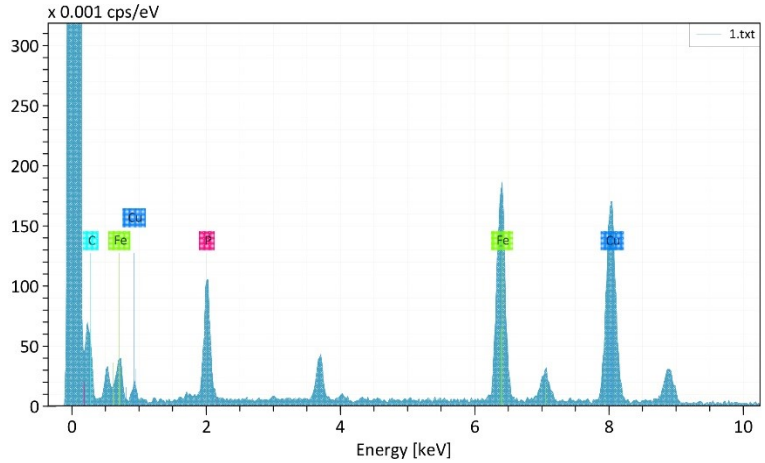

Figure S1. TEM-associated energy-dispersive X-ray spectroscopy (EDS) pattern of FeP nanoparticles.

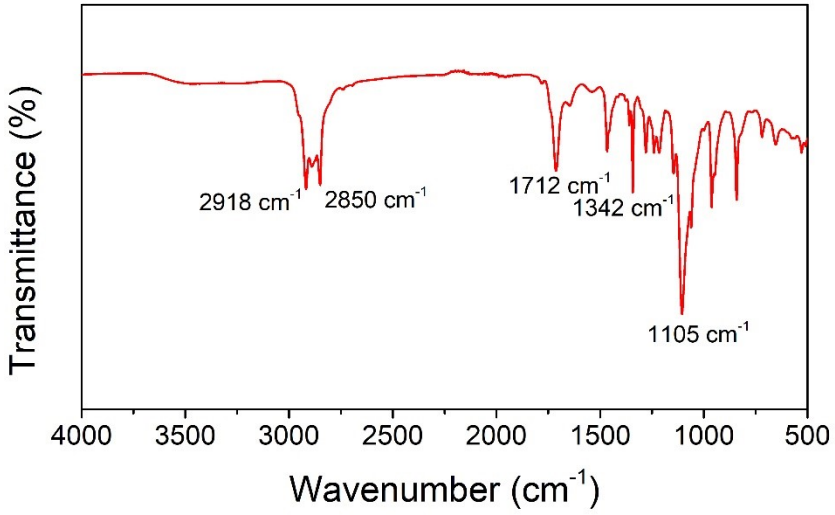

Figure S2. FTIR spectrum of FeP nanoparticles.

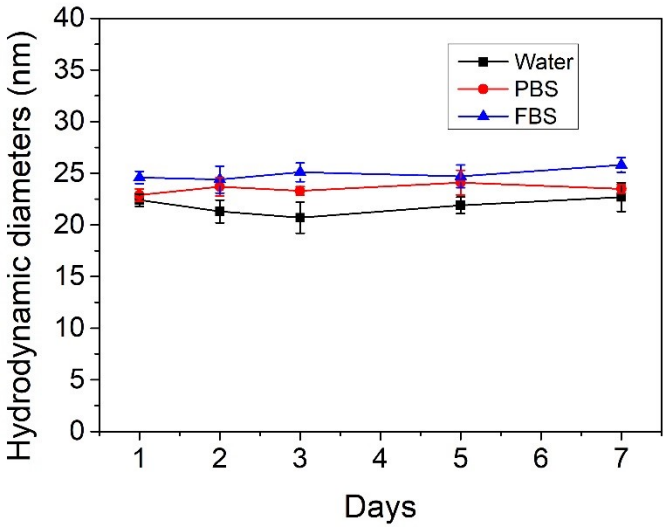

Figure S3. Hydrodynamic diameter distributions over 7 days of FeP nanoparticles in water, phosphate buffered saline (PBS), and 10% fetal bovine serum (FBS), respectively.

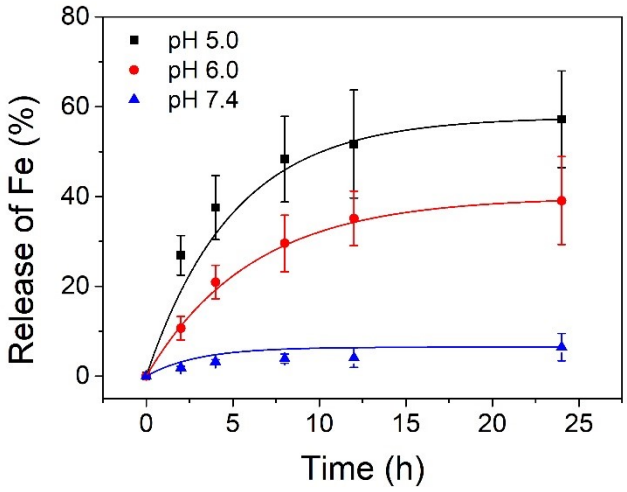

Figure S4. Release profiles of Fe ions from FeP nanoparticles at pH 5.0, pH 6.0, and pH 7.4, respectively.

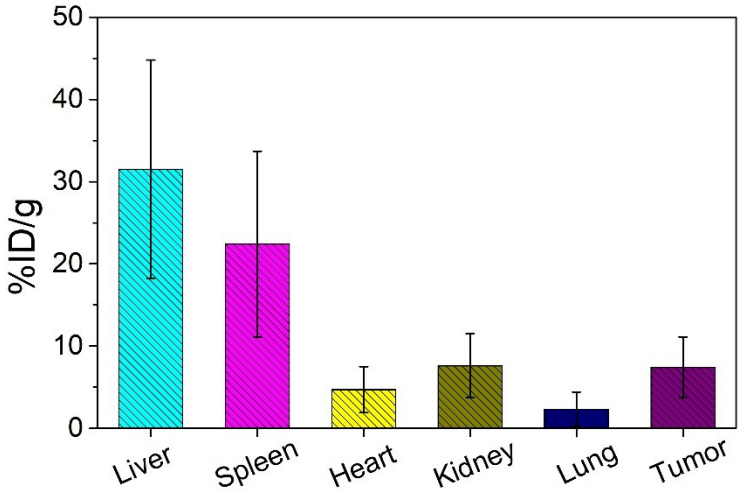

Figure S5. Biodistribution of FeP nanoparticles in mice 10 h after the injection (n = 5)

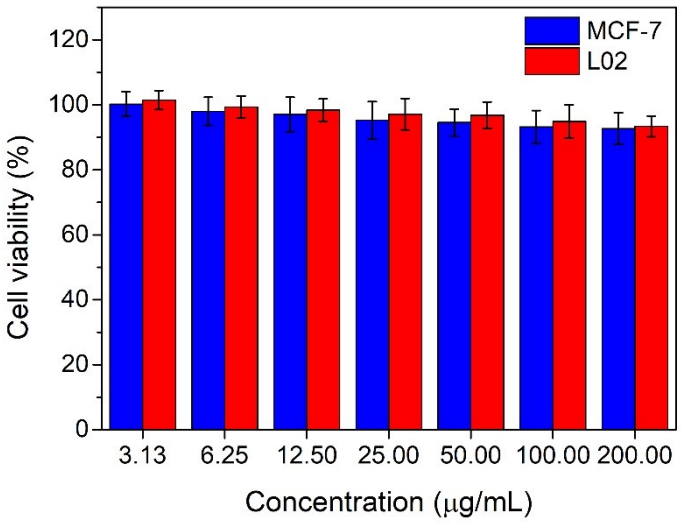

Figure S6. Cell viability of MCF-7 and L02 cells after incubated with FeP nanoparticles with different concentrations at 37 °C for 24 h.