Electronic Supplementary Information

Highly magnetic iron carbide nanoparticles as effective $T_2$ contrast agents

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1. Supplementary Figures

![Figure S1. C 1s XPS spectrum of Fe$_5$C$_2$ nanoparticles.](image_url)

**Figure S1.** C 1s XPS spectrum of Fe$_5$C$_2$ nanoparticles.
Figure S2. (a) Low-magnification and (b) high-magnification TEM images (inset, photograph) of the water-dispersible Fe₅C₂ nanoparticles.

Figure S3. (a) The dynamic light scattering (DLS) analysis of tartrate-coated Fe₅C₂ nanoparticles dispersed in PBS. The hydrodynamic diameter (HD) of Fe₅C₂ nanoparticles is about 40 nm. (b) The analysis of measured HD values over at least 18 days, indicating the good distribution and stability of Fe₅C₂ nanoparticles in aqueous solution.
**Figure S4.** Characterizations of Fe₃O₄ nanoparticles and amor-Fe nanoparticles. (a, d) TEM images, (b, e) XRD patterns, and (c, f) magnetic hysteresis loops recorded at 300 K of Fe₃O₄ nanoparticles and amor-Fe nanoparticles, respectively. TEM images show that the size of Fe₃O₄ nanoparticles and amor-Fe nanoparticles are ~20 and ~14 nm in diameter, respectively. XRD patterns confirm the crystal structure of Fe₃O₄ nanoparticles and amor-Fe nanoparticles. Magnetic hysteresis loops reveal that the saturation magnetization values of Fe₃O₄ nanoparticles and amor-Fe nanoparticles are 53.44 and 26.08 emu g⁻¹ at 300 K, respectively.

**Figure S5.** The linear fitting of relaxation rates versus Fe concentrations for Fe₅C₂ nanoparticles and Fe₃O₄ nanoparticles at 7 T, respectively.
**Figure S6.** T₂-weighted *in vivo* MRI images of mice (coronal plane) collected at different time points after intravenous injection of Fe₅C₂ nanoparticles and Fe₃O₄ nanoparticles (with a dose of 2.0 mg Fe/kg of mouse body weight), respectively. The regions of liver were indicated by dash lines.

### 2. Experimental Sections

#### Preparation of Fe₅C₂ nanoparticles

We prepared Fe₅C₂ nanoparticles according to the method described in the previous paper with some modifications.¹ In a typical procedure, a mixture containing 7.0 g of octadecylamine, 0.8 g of CTAB, and 2 mL of oleylamine was mechanically stirred and heated to 180 °C. Under a blanket of Ar, 0.2 mL of Fe(CO)₅ (1.5 mmol) was injected. After 10 min, the solution was further heated to 350 °C and kept at this temperature for 30 min before it was cooled down to room temperature. The product was washed with ethanol and hexane several times to remove the excess octadecylamine. Finally, the Fe₅C₂ nanoparticles were dispersed in hexane for further use.

#### Preparation of 14 nm amor-Fe nanoparticles

We prepared amor-Fe nanoparticles according to the method described in the previous paper.² In a typical procedure, 10 mL of 1-octadecene containing 0.2 mL of oleylamine was mechanically stirred and heated to 180 °C. Under a blanket of Ar, 0.2 mL of Fe(CO)₅ (1.5 mmol) was injected. The mixture was kept at this temperature for 20 min before it was cooled down to room temperature. The products was precipitated by adding of isopropanol, then collected by centrifugation and redispersed in hexane for further use.
Preparation of 20 nm Fe₃O₄ nanoparticles

We prepared Fe₃O₄ nanoparticles according to the method described in the previous paper. In a typical procedure, 44 g of the iron-oleate complex and 8 mL of oleic acid were dissolved in 310 mL of 1-octadecene at room temperature. The reaction mixture was subsequently heated to reflux (~315 °C) for 1.5 h before it was cooled down to room temperature. The products was precipitated by adding of ethanol, then collected by centrifugation and redispersed in hexane for further use.

Preparation of water soluble nanoparticles

To make the nanoparticles dispersible in aqueous media for biomedical applications, we developed a simple but efficient ligand-exchange method using sodium tartaric as a phase transfer agent. Generally, 16 mL of tetrahydrofuran containing ~2 mg nanoparticles (e.g., Fe₅C₂, Fe₃O₄, and amor-Fe) were added into 8 mL of water containing 15 mg sodium tartaric and 33 mg NaHCO₃. The mixture was then heated to 95 °C and mechanically stirred for 4 h. After being cooled to room temperature, the mixture was centrifuged (7000 rpm, 10 min) to collect products. The final products were redispersed in water and then filtered through sterilized membrane filters (pore size 0.22 µm) for further use.

Cytotoxicity assay

The cytotoxicity of the Fe₅C₂ nanoparticles was tested by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. HeLa cells were firstly seeded into a 96-well plate with a density of 1×10⁴ cells/well in DMEM, and incubated in the atmosphere of 5% CO₂ at 37 °C for 24 h. The cells were then incubated with Fe₅C₂ nanoparticles at various Fe concentrations (0.39, 0.78, 1.56, 6.25, 12.5, 25, 50 and 100 µg/mL) for another 24 h. Subsequently, the culture medium was removed, each well was added with 100 µL new culture medium containing MTT (0.5 mg/mL) and the plate was incubated for 4 h at 37 °C. Then the medium was discarded and each well was added 200 µL DMSO. The OD₄₉₀ value (Abs.) of each wells were measured by a MultiSkan FC microplate reader immediately. Cell viability was calculated from OD₄₉₀ value of experimental group by subtracting that of blank group.
Transverse relaxivity ($r_2$) and MRI phantom studies

A series of desired nanoparticles aqueous solution (Fe$_5$C$_2$, Fe$_3$O$_4$, and amor-Fe) containing 1% agar with different Fe concentrations (400, 200, 100, 50, and 25 µM) were prepared for MRI phantom and transverse relaxivity ($r_2$) studies. All experiments were performed on a 0.5 T NMR120-Analyst NMR system (Niumag Corporation, Shanghai, China). The transverse relaxation times ($T_2$) were measured by a Carr-Purcell-Meiboom-Gill (CPMG) sequence and then were used for calculating the transverse relaxation rates ($R_2$, i.e., $1/T_2$) of the samples. The relaxivity ($r_2$) was determined from the slope of the plot of $1/T_2$ against iron concentration ([Fe], in mM). $T_2$-weighted phantom images were acquired using a 2D multi-slice spin-echo (MSE) sequence with the following parameters: repetition time (TR) = 2000, echo time (TE) = 40 ms, $512 \times 512$ matrices, slices = 1, thickness = 1 mm, NS = 4.

In vivo MRI study

Animal experiments were executed according to the protocol approved by Institutional Animal Care and Use Committee of Xiamen University. Healthy BALB/c mice weighing 20-30 g were chosen for in vivo MRI studies. $T_2$-weighted images containing transverse and coronal planes were first collected on a 7 T MRI scanner (Varian 7 T Micro MRI System) without injection of nanoparticles. The mice were then intravenously injected with 100 µL of Fe$_5$C$_2$ or Fe$_3$O$_4$ aqueous solution (dosage of 2.0 mg Fe per kg of mouse body weight). The same slices were further acquired at different time points (0.5 h, 1 h, 2 h, 4 h and 24 h) after the injection. All the images were obtained using a fast spin-echo multislice sequence (fSEMS) sequence under the following parameters: TR/TE = 3000/40 ms, $256 \times 256$ matrices, slices = 5, thickness = 2 mm, averages = 2, FOV = $80 \times 80$. To quantify the contrast enhancement, the signal-to-noise ratio (SNR) was measured by finely analyzing regions of interest (ROIs) of the transverse images, and the contrast enhancement was defined as the decrease of SNR after the injection, $\Delta \text{SNR} = (|\text{SNR}_{\text{post}} - \text{SNR}_{\text{pre}}|)/\text{SNR}_{\text{pre}}$.

Characterization

Transmission electron microscope (TEM) and high-resolution TEM (HRTEM) images were carried out on a JEM-2100 transmission electron microscope at an accelerating voltage of 200 kV. The X-ray
diffraction (XRD) patterns were measured on a Panalytical X’pert PRO. The X-ray photoelectron (XPS) data was collected on a PHI Quantum 2000 XPS spectrometer. The hysteresis loops (at 300 K) were recorded on a Quantum Design MPMS-XL-7 system. The element analysis of Fe in the samples was carried out by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer nano ZS instrument.

References