Electronic Supplementary Information

Highly magnetic iron carbide nanoparticles as effective T_2 contrast agents

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1. Supplementary Figures

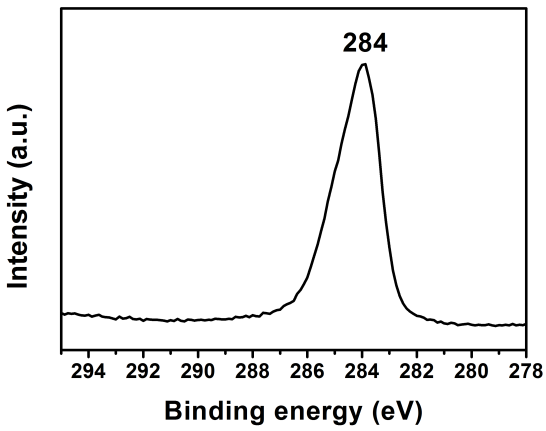

Figure S1. C 1s XPS spectrum of Fe_5C_2 nanoparticles.

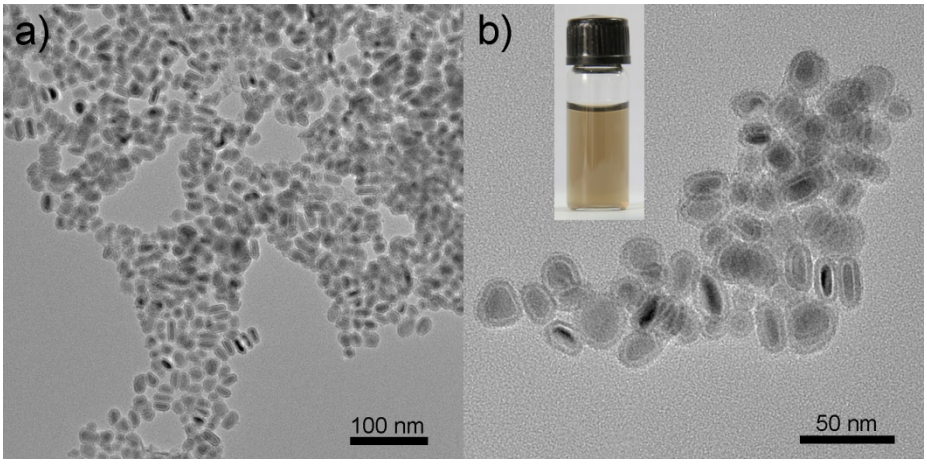

Figure S2. (a) Low-magnification and (b) high-magnification TEM images (inset, photograph) of the water-dispersible Fe_5C_2 nanoparticles.

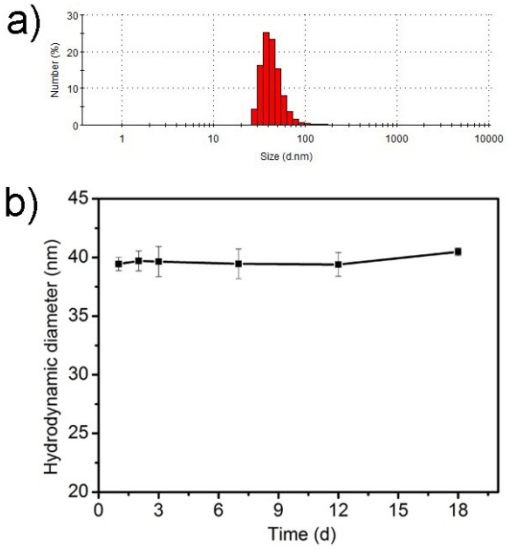

Figure S3. (a) The dynamic light scattering (DLS) analysis of tartrate-coated Fe_5C_2 nanoparticles dispersed in PBS. The hydrodynamic diameter (HD) of Fe_5C_2 nanoparticles is about 40 nm. (b) The analysis of measured HD values over at least 18 days, indicating the good distribution and stability of Fe_5C_2 nanoparticles in aqueous solution.

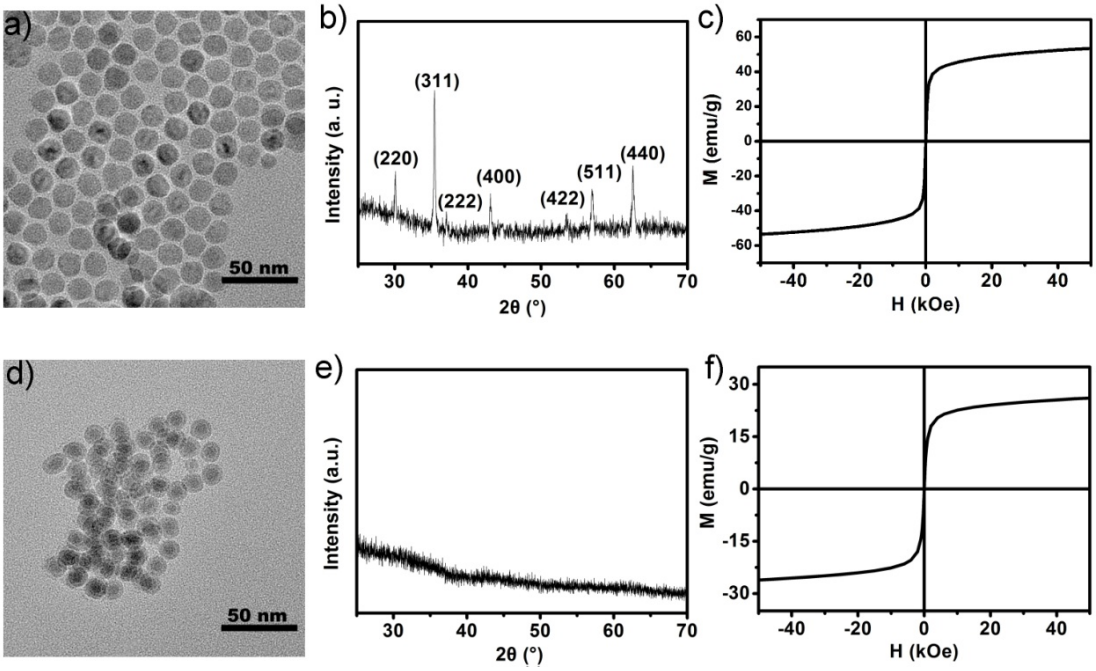

Figure S4. Characterizations of Fe_3O_4 nanoparticles and amor-Fe nanoparticles. (a, d) TEM images, (b, e) XRD patterns, and (c, f) magnetic hysteresis loops recorded at 300 K of Fe_3O_4 nanoparticles and amor-Fe nanoparticles, respectively. TEM images show that the size of Fe_3O_4 nanoparticles and amor-Fe nanoparticles are ~20 and ~14 nm in diameter, respectively. XRD patterns confirm the crystal structure of Fe_3O_4 nanoparticles and amor-Fe nanoparticles. Magnetic hysteresis loops reveal that the saturation magnetization values of Fe_3O_4 nanoparticles and amor-Fe nanoparticles are 53.44 and 26.08 emu g^{-1} at 300 K, respectively.

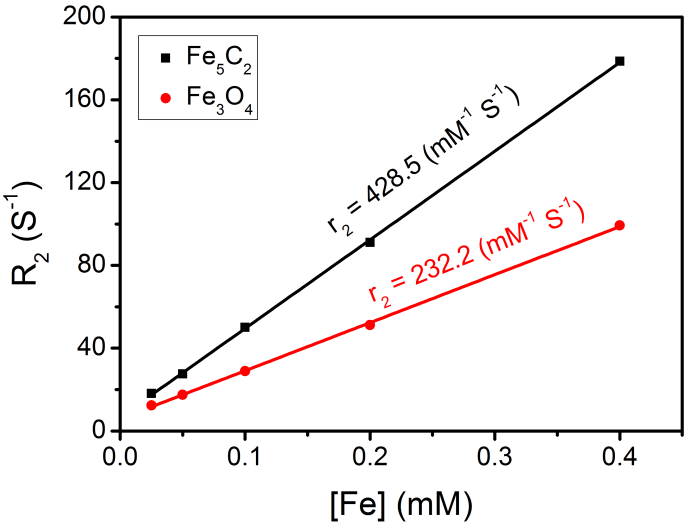

Figure S5. The linear fitting of relaxation rates versus Fe concentrations for Fe_5C_2 nanoparticles and Fe_3O_4 nanoparticles at 7 T, respectively.

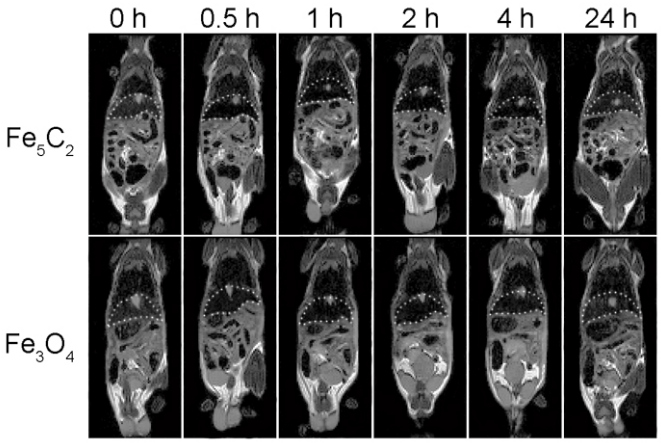

Figure S6. T_2-weighted *in vivo* MRI images of mice (coronal plane) collected at different time points after intravenous injection of Fe_5C_2 nanoparticles and Fe_3O_4 nanoparticles (with a dose of 2.0 mg Fe/kg of mouse body weight), respectively. The regions of liver were indicated by dash lines.

2. Experimental Sections

Preparation of Fe_5C_2 nanoparticles

We prepared Fe_5C_2 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 Fe_5C_2 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 Fe_3O_4 nanoparticles

We prepared Fe_3O_4 nanoparticles according to the method described in the previous paper.[3] 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., Fe_5C_2, Fe_3O_4, and amor-Fe) were added into 8 mL of water containing 15 mg sodium tartaric and 33 mg $NaHCO_3$. 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 Fe_5C_2 nanoparticles was tested by 3-(4, 5-dimethylthiazol-2-y1)-2, 5-diphenyltetrazolium bromide (MTT) method. HeLa cells were firstly seeded into a 96-well plate with a density of 1×10^4 cells/well in DMEM, and incubated in the atmosphere of 5% CO_2 at 37 °C for 24 h. The cells were then incubated with Fe_5C_2 nanoparticles at various Fe concentrations (0.39, 0.78, 1.56, 3.13, 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 OD_{490} value (Abs.) of each wells were measured by a MultiSkan FC microplate reader immediately. Cell viability was calculated from OD_{490} value of experimental group by substracting that of blank group.

Transverse relaxivity (r_2) and MRI phantom studies

A series of desired nanoparticles aqueous solution (Fe_5C_2, Fe_3O_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 × 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_5C_2 or Fe_3O_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 × 256 matrices, slices = 5, thickness = 2 mm, averages = 2, FOV = 80 × 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, $\Delta SNR = (|SNR_{post} - SNR_{pre}|)/SNR_{pre}$.

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

1. C. Yang, H. Zhao, Y. Hou and D. Ma, *J. Am. Chem. Soc.*, 2012, **134**, 15814-15821.
2. S. Peng, C. Wang, J. Xie and S. Sun, *J. Am. Chem. Soc.*, 2006, **128**, 10676-10677.
3. J. Park, K. An, Y. Hwang, J. G. Park, H. J. Noh, J. Y. Kim, J. H. Park, N. M. Hwang and T. Hyeon, *Nat. Mater.*, 2004, **3**, 891-895.