Supporting Information

Controlled Synthesis of $MnFe_2O_4$ Nanoparticles and Gd Complex-Based Nanocomposites as Tunable and Enhanced T1/T2-Weighed MRI Contrast Agents

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Chemicals. Iron (III) acetylacetonate (99.9%), manganese (II) acetylacetonateoleic, oleic acid, polyethylene glycol (MW=4000), 1,ω-diaminopolyoxyethylene (MW=3350), Folic acid, 3,4-Dihydroxybenzaldehyde, diethylenetriamine pentaacetic anhydride (DTPAA), dicyclohexylcarbodiimide (DCC) and hydroxysuccinimide (NHS) were purchased from Sigma Aldrich. All the dialysis bags (MWCO 8000-14000) were obtained from Shanghai Med.

Synthesis of 16 nm $MnFe_2O_4$ NPs.

4 mmol of $Fe(acac)_3$ and 2 mmol of $Mn(acac)_2$ was dissolved in 15 mL of oleic acid. The reaction mixture was dehydrated at 120 ^{0}C for 1 h under N_2 atmosphere, then quickly heated to 330 ^{0}C, and aged at this temperature for 4 h. After the reaction, the solution was allowed to cool down to room temperature. The $MnFe_2O_4$ NPs were precipitated upon the addition of 50 mL of isopropyl alcohol and centrifuged. In order to remove the excess oleic acid on the surface of NPs, the NPs were washed by petroleum ether and ethanol mixed solution. Finally, the product was dispersed in hexane.

Synthesis of 18 and 27 nm $MnFe_2O_4$ NPs.

Under identical conditions, the 18 nm $MnFe_2O_4$ NPs and 27 nm $MnFe_2O_4$ NPs were synthesised. The only difference is that 18 nm $MnFe_2O_4$ NPs corresponds to 2 mmol of $Fe(acac)_3$ and 1 mmol of $Mn(acac)_2$, while 27 nm $MnFe_2O_4$ NPs corresponds to 8 mmol of $Fe(acac)_3$ and 4 mmol of $Mn(acac)_2$. Each $MnFe_2O_4$ NPs were precipitated upon the addition of 50 mL of isopropyl alcohol and centrifuged. In order to remove the excess oleic acid on the surface of NPs, the NPs was washed by petroleum ether and ethanol mixed solution. Finally, the product was dispersed in hexane.

Synthesis of EDA-Derivatized Folic acid.

Folic acid (0.441 g, 1 mmol) was dissolved in 20 mL of dry dimethyl sulphoxide (DMSO) to which 0.246 g (1.2 mmol) of dicyclohexylcarbodiimide (DCC) and 0.23 g (2.0 mmol) of hydroxysuccinimide (NHS) were added. The reaction mixture was stirred for 14 hours at room temperature in the dark. The byproduct, dicyclohexylurea, was filtered off. The filtrate was mixed with 0.160 g (1.0 mmol) N-tert-Butoxycarbonyl-1, 2-ethanediamine and allowed to react at 25 °C for 14 hours and subsequently filtrated. The filtrate was poured into 50 mL of diethylether, and the resulting yellow precipitate was filtrated. After thoroughly washing with cold diethylether, the yellow solid was dissolved in acetonitrile and precipitated again, pouring in diethylether. After drying in vacuum, the above yellow solid were mixed with 5 mL of TFA (trifluoracetic acid) and stirred for 2 h at 40 °C in a water bath. TFA was removed under reduced pressure and the remaining brownish gel was dissolved in 50 mL of water. The solution was poured into 800 mL of acetonitrile and a brownish precipitate was collected via filtration. The solid was dried under reduced pressure for 18 h at room temperature. Yield:60%. MS: m/z = 482 $[M+H]^+$. ^1H NMR (DMSO-d6, 300 MHz, δ in ppm): 186−1.99 (m, 2H, C21-H), 2.01−2.06 (m, 2H, C22-H), 2.21−2.38 (m, 2H, C25-H), 2.45 (m, 2H, C26-H), 4.34 (dd, J=5.4, 9.36 Hz, 1H, C19-H), 4.49 (s, 2H, C9-H_2), 6.66 (d, J=8.7Hz, 2H), 7.66 (d, J=8.7Hz, 2H), 8.65 (s, 1H).

Synthesis of Folate-DTPA-NH-PEG-DIB (1a).

To a solution of CaDTPA (35.7 mg, 0.1m mol) in 5 mL of DMF was added dropwise EDA-derivatized folic acid (48.4 mg, 0.1mmol) in 2 mL of DMF. After the mixture solution became clearly, the reaction mixture was added dropwise to a solution of DIB-PEG-NH2 (400 mg 0.1 mmol) in 5 mL anhydrous dichloromethane, and continued to stir 12 hours. The product was precipitated by adding diethyl ether, and collected by centrifugation at 4000 rpm. After washed with DMSO and diethyl ether (1/10, v/v) 3 times, the product was dried in vacuum for 24 h.

Synthesis of Gd: Folate -DTPA-NH-PEG-DIB (1b).

The **1a** (90 mg) and the $GdCl_3$ (9 mg) were added together in DMF (10mL). The mixtures were stirred at 25 °C for 24 hours. The product was precipitated by adding diethyl ether, and collected by centrifugation at 4000 rpm. After washed with DMF, diethyl ether (1/10, v/v) 3 times, the product was dried in vacuum for 24 hours.

Synthesis of Gd: Folate -DTPA-NH-PEG-DIB-$MnFe_2O_4$ (1c).

0.02 g of $MnFe_2O_4$ NPs was added into 10 mL of $CHCl_3$ containing 0.06 g **1b**. The mixture was stirred 12h in the dark at room temperature. The particles were precipitated upon the addition of 10 mL of hexane and centrifuged. After washed with $CHCl_3$ and hexane (1:5 V/V), the particles was then dispersed in water and dialysised with H_2O for 24 h to remove unreacted organic molecules.

Cytotoxicity assay.

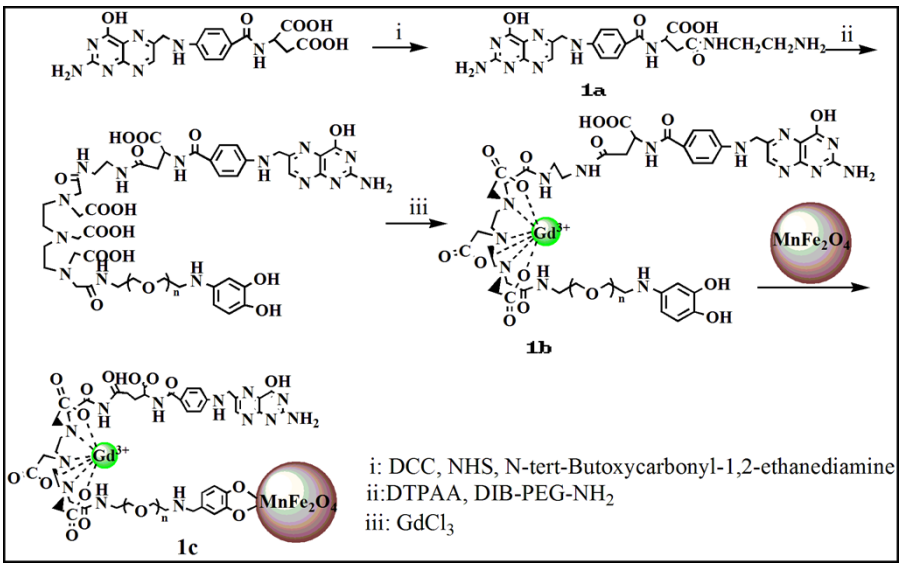
In vitro cytotoxicity of the **1c** was evaluated by performing methyl thiazolyl tetrazolium (MTT) assay of the HeLa cells incubated with the particles. Cells were seeded into a 96-well cell culture plate with a density of 5×10^4 cells/well in DMEM with 10% FBS at 37 ^{0}C under 5% CO_2 for 24 h. Then, the cells were incubated with the **1c** with different concentrations (0, 20, 40, 80, 160 μg/mL in DMEM) for 48h and 72 h respectively at 37 ^{0}C under 5% CO_2. Thereafter, MTT (20 mL, 5 mg/mL) was added to each well and the plate was incubated for 4 h at 37 ^{0}C. After the addition of dimethyl sulfoxide (DMSO, 100 μL/well), the cell plate was allowed to stand at 37 ^{0}C for 15 min. The optical density was measured at 492 nm using a microplate reader (Shanghai Sanco Instrument Co., Ltd. 318C-microplate reader).

Cell MRI.

Hela cells and A549 cells (4×10^6) were incubated with **1c** with different concentrations for 1.5 h at 37 ^{0}C. After incubation, the cells were washed with PBS buffer three times and redispersed in PBS buffer with a cell density of 1×10^6 cells/mL before MR imaging. All MR imaging measurements were performed with a 0.55 T systems (0.557 T Siemens Magnetom Trio). T1-weighted MR images were acquired using a conventional spin-echo sequence under the following parameters: TR/TE = 500/12 ms, 220 ×320 matrices, 82 × 120 mm field of view, 140 Hz/Px of bandwidth, a slice thickness of 3 mm. T2-weighted MR images using a fast spin-echo sequence was used to reduce acquisition time under the following parameters: TR/TE=3600/90 ms, 220×320 matrices, 82×120 mm field of view, 220 Hz/Px of bandwidth, and a slice thickness of 3 mm.

Histopathologic and Immunohistochemical Assessments.

Mice were intravenously injected with 5 mg/kg nanoparticles for 15 days and underwent physical evaluations. Portions of liver, spleen, Muscle, and kidney tissue were fixed in 3.7% formaldehyde at room temperature and then embedded in paraffin blocks. Tissue sections 5 μm thick were stained with hematoxylin and eosin. The morphology of the tissue was observed under a microscope at 10× and 20× magnification.



Scheme S1. Synthetic route of Gd: Folate -DTPA-NH-PEG-DIB-$MnFe_2O_4$-DIB-PEH-NH-FITC **(1c) .**

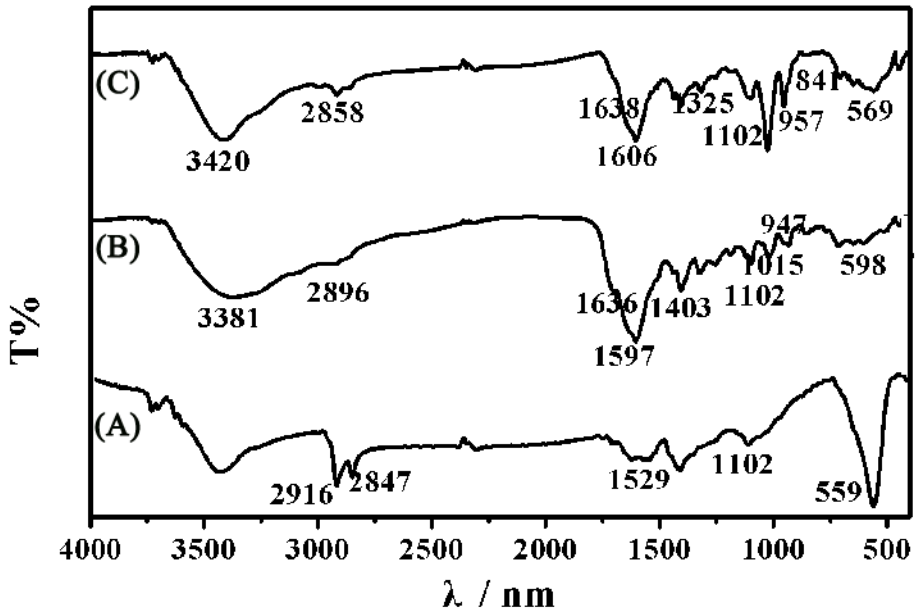


Fig S1. The IR spectra of (A) as-synthesized $MnFe_2O_4$ NPs ; (B) Gd: Folate -DTPA-NH-PEG-DIB; (C) Gd: Folate -DTPA-NH-PEG-DIB-$MnFe_2O_4$ NPs.

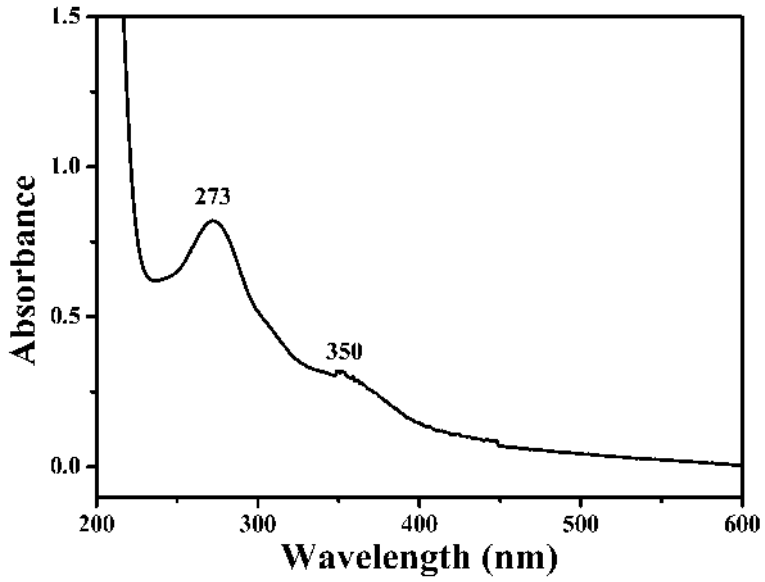


Fig S2. UV-vis detection of **1c**. The absorbance peaks at 273 nm and 350 nm belong to folic acid (FA).

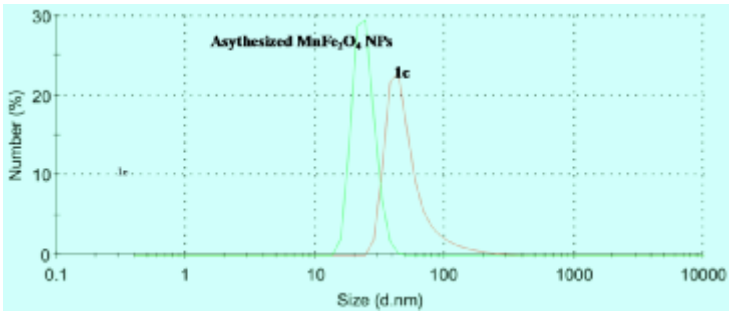


Fig S3. Hydrodynamic diameters measured by DLS for the as-synthesized $MnFe_2O_4$ NPs dispersed in hexane and the **1c** dispersed in PBS.

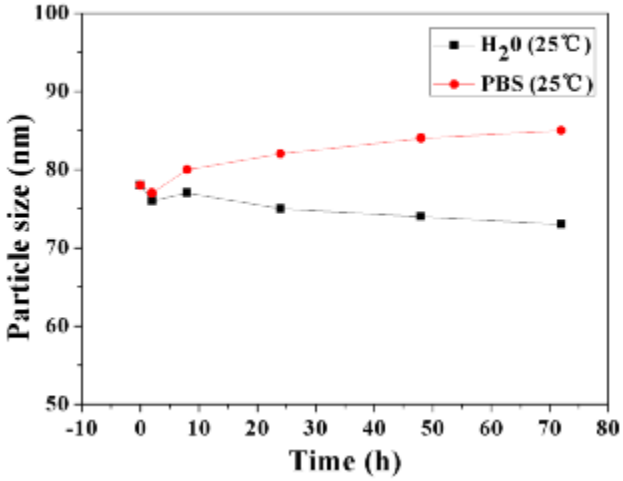


Fig S4.The hydrodynamic diameters of the **1c** dispersed in PBS and H_2O for 72 h.

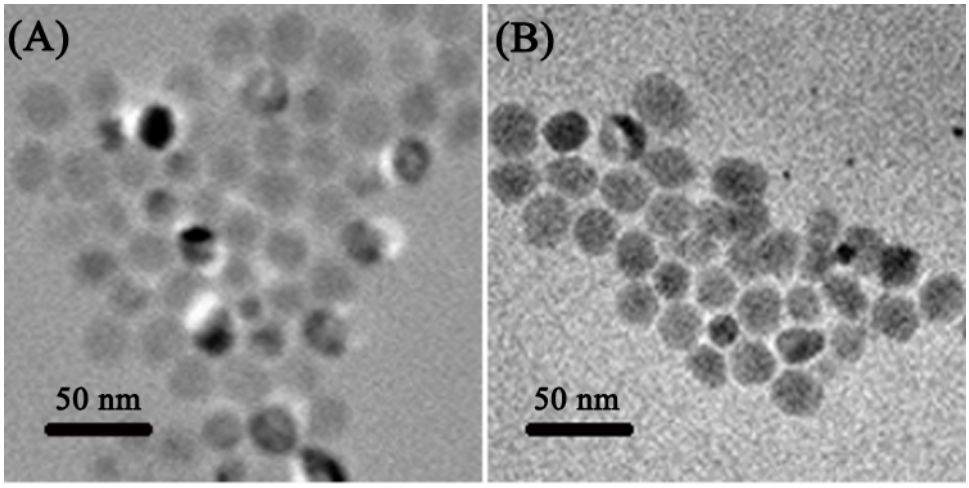


Fig S5. (A) TEM images of $MnFe_2O_4$ in hexane and (B)**1c** dispersed in H_2O.

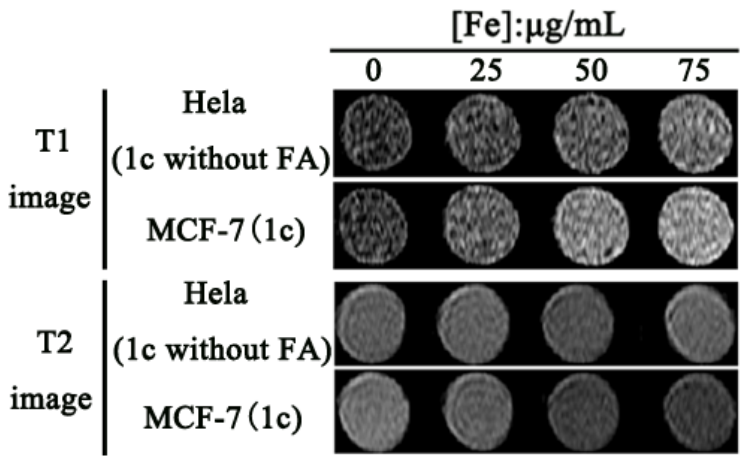


Fig S6. T1/T2-weighted MR images of **1c** without FA in Hela cells and **1c** in MCF-7 cells at different concentrations of Fe after incubation for 1.5 h on the 0.55T MR system.