Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information for:

Monodisperse Water-soluble Magnetite Nanoparticles Prepared by Polyol Process for High-performance Magnetic Resonance Imaging

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Synthesis of Magnetite nanoparticles: Water-soluble magnetite nanoparticles were prepared via our previously published approach. In a typical synthesis procedure, $Fe(acac)_3$ (1 mmol, 99%, Acros) and TREG (30 ml, 99%, Aldrich) was mixed and slowly heated to reflux (～278 ℃) and kept at reflux for 30 min under argon protection giving a black homogeneous colloidal suspension. After cooling down to room temperature, 20 ml of ethyl acetate was added to the reaction solution resulted in a black precipitation of magnetite nanoparticles which was then separated from the solution by a magnetic field. After washed with ethyl acetate for three times, the precipitation was re-dispersed in polar solvents such as ethanol and water for further investigation. The solid productions were obtained by drying the precipitation under vacuum.

Characterization of magnetite nanoparticles: Transmission electron microscope (TEM), high-resolution TEM (HRTEM) images and selected area electron diffraction (SAED) patterns were obtained on a JEOL JEM 2010 electron microscope at an accelerating voltage of 200 kV. Samples for TEM analysis were prepared by spreading a drop of as-prepared products dilute dispersion on amorphous carbon-coated copper grids and then dried in air. X-ray powder diffraction (XRD) patterns of the products were recorded with a Rigaku D/max-γB diffractometer equipped with a rotating anode and a Cu Kα source (λ=0.154056 nm). Magnetic measurements were carried out using the physical properties measurement system (PPMS) of Quantum Design with a magnetic field up to 5 T. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin-Elmer Spectrum One spectrometer. Thermogravimetric analyses (TGA) were performed under nitrogen at a heating rate of 5 /min from room temperature up to 550 using a NETZSCH STA 449 C analyzer X-Ray Photoelectron Spectroscopy (XPS) studies were performed on a PHI 5300 ESCA system with a hemispherical analyzer and an Al Kα radiation (1486.6 eV). Dynamic light scattering (DLS) measurements were performed with a Malvern 3000HSA Zetasizer, provided with a He/Ne laser of 633 nm wavelength. The iron concentrations were quantified using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Perkin–Elmer Optima 5300 DV).

Cell culture: Rat C6 glioma cell lines were kindly provided by Kawamoto K (Dept. of Neurosurgery, Kansai Medical University, Osaka, Japan), and were routinely cultured at 37 °C in a humidified CO_2 atmosphere in DMEM supplemented with 10% fetal bovine serum, 100 kU/L penicillin, and 100 mg/L streptomycin. Cell viability and density were determined through staining with Trypan Blue. Cell counts were obtained using a hemocytometer.

Neural cells were prepared from the cortex of newborn Wistar rats in accordance with methods described previously.[1] In brief, cortical tissue was carefully freed from blood vessels and meninges. Tissue was trypsinized for 20 minutes, carefully disintegrated with a fire-polished pipette, and washed twice. The cortical cells were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum, 100 kU/L penicillin, 100 mg/L streptomycin, and 2% B-27 Supplements, and the medium was changed every third day.

Nanoparticles uptake: 24 h after seeding, C6 glioma cells and normal neural cells were cultured with magnetite nanoparticles at concentration of 100 mg/L for 4 h. Following labelling, the samples were washed twice with cell culture medium and twice with PBS buffer. For Prussian blue staining, cells were fixed with 4 % paraformaldehyde solution and washed with PBS buffer, followed by the incubation in perl's solution (equal parts of 6 % hydrochloric acid and 2 % Potassium ferrocyanide) for 30 min. After washed with deionized water,

they were counterstained with Nuclear Fast Red solution. The specimen were then mounted and examined under a light microscope.

MR imaging procedure: The experiments were performed in a clinical 1.5 T MRI scanner (Signa Excite HDe, GE Health Care, Milwaukee, WI) using a surface coil. Magnetite nanoparticles were dispersed in water at various Fe concentrations (1, 1/2. 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, and 0 mM), and 2×10^6 cells in 100 μL were suspended in 4% agarose. All specimen were loaded into 24-well plates at volume of 3ml per well. The spaces surrounding each well were full of deionize water to allow appropriate image acquisition. T_1 map was acquired using inversion recovery, fast spin-echo imaging sequence (*Inversion Time*=100, 200, 300, 400, 500 ms, T_R=4000 ms, T_E=4 ms, acquisition matrix=256×256, field of view=18×18 cm, slice thickness=6 mm, number of averages=1, ETL=4). T_2 map was acquired using spin-echo imaging sequence (T_R=2000 ms, T_E=30, 60, 90, 120 ms, acquisition matrix=256×256, field of view=18×18 cm, slice thickness=6 mm, number of averages=1). T_1 and T_2 measurements were performed via a nonlinear least-squares fit to changes in the mean signal intensity within each well as a function of T_R or T_E using the provided quantification software. Finally, the r_1 and r_2 relaxivity values were determined through the least-square curve fitting of $1/T_1$ and $1/T_2$ versus the iron concentrations of the nanoparticles.

In vitro cell cytotoxicity study: The MTT assay was performed to evaluate whether the magnetite nanoparticles impairs viability of glioma C6 cells and neural cells using corresponding untreated cells as control. Cells were seeded into 96-well plates at densities of 6 × 103 cells per well for 24 h. Then magnetite nanoparticles at final concentration of 10, 25, 50, 100, 150, 100, and 200 mg/L (iron concentrations) were added to cells for 24 h. The cells were washed twice with PBS and replenished with fresh medium, followed by incubation for a further 48 h. Then, cells were examined by MTT assay.[2]

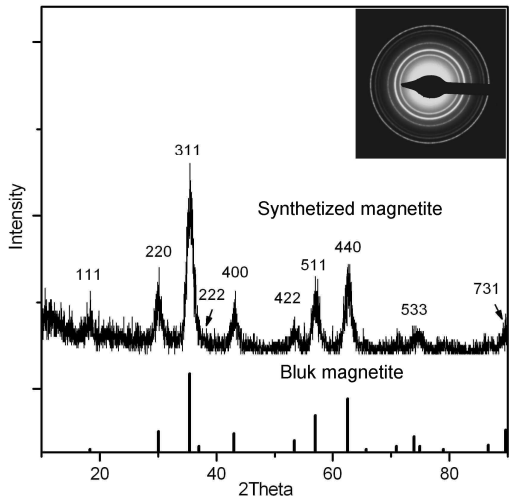

Fig. S1 X-ray diffraction patterns for as-synthesized Fe_3O_4 nanoparticles and standard XRD data for magnetite (JCPDS No. 19-0629); the inset is the SAED pattern of the Fe_3O_4 nanoparticles.

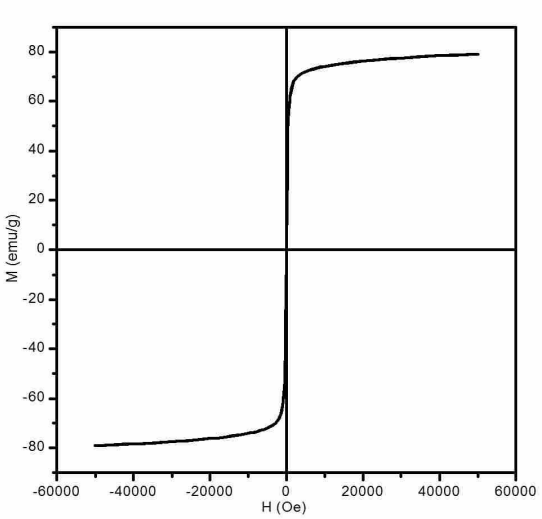

Fig. S2 The hysteresis loop of the Fe_3O_4 nanoparticles at 300 K.

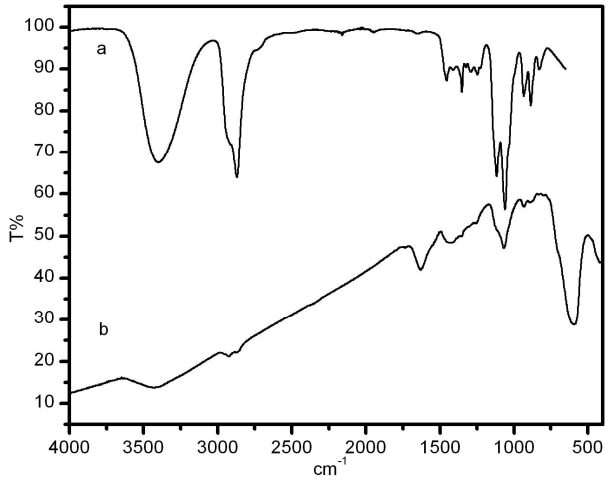

Fig. S3 FT-IR spectra of TREG (a) and as-synthesized Fe_3O_4 nanoparticles (b).

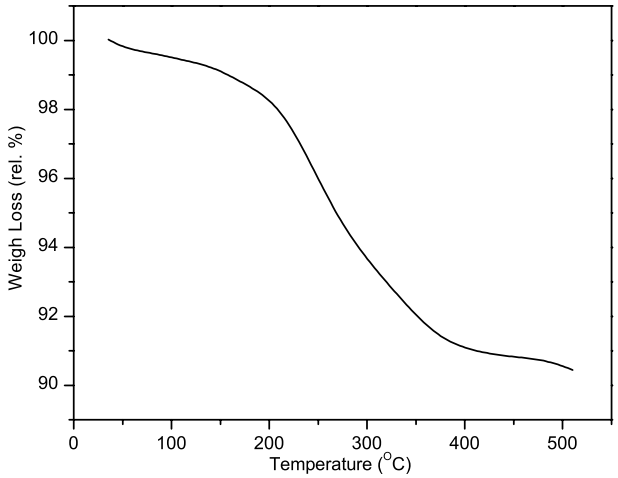

Fig. S4 TGA curve of the Fe_3O_4 nanoparticles.

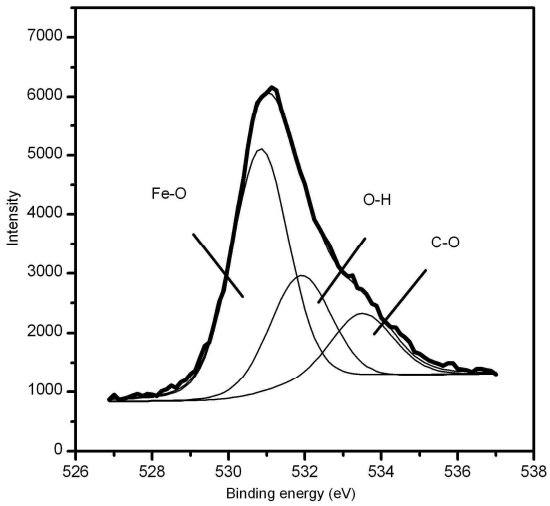

Fig. S5 The O1s high resolution X-ray photoelectron spectra of the Fe_3O_4 nanoparticles. Three peaks fitted at 530.2, 531.6 and 533.2 eV could be assigned to oxygen in a Fe-O (lattice oxygen) component of magnetite, oxygen in an O-H component of surface hydroxyl and oxygen in a C-O component of TREG, respectively.

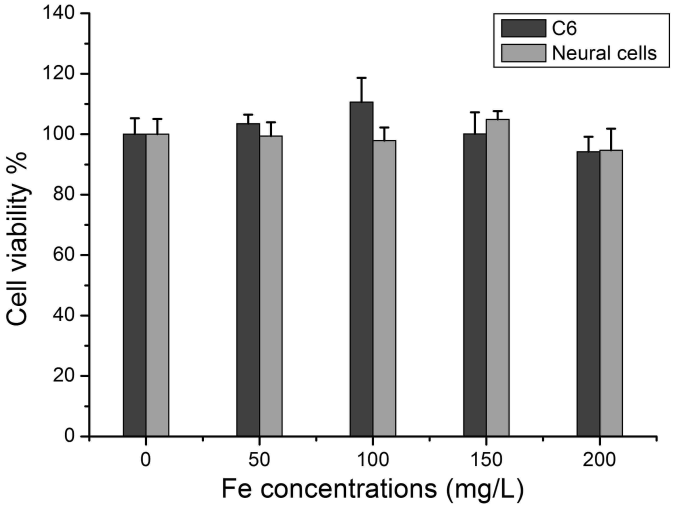

Fig. S6 Cytotoxicity profile of as-prepared Fe_3O_4 nanoparticles via MTT assay on C6 and neural cells.

1 X. Yu, L. An, *Cell Mol. Neurobiol*., 2002, **22**, 197.

2 T. Mosmann, *J. Immunol. Methods*, 1993, **95**, 55.