Supporting Information for

Mesoporous Silica-coated Hollow Manganese Oxide Nanoparticles as Positive T1 Contrast Agents for Labeling and MRI Tracking of Adipose-Derived Mesenchymal Stem Cells

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Materials. Manganese chloride tetrahydrate (98%), 1-octadecene (90%), ammonium hydroxide (29.4%), cyclohexane (99.0%), oleic acid (90%), rhodamine B isothiocyanate (RITC), 3-aminopropyltriethoxysilane (APTES), and Igepal CO-520 were purchased from Aldrich Chemicals. Sodium oleate was purchased from TCI Chemicals. Cetyltrimethylammonium bromide (CTAB) and tetraethylorthosilicate (TEOS) were purchased from Acros Organics. Ammonium hydroxide (28.0~30.0%), ethylacetate (99%), hydrochloric acid (35~37%), n-hexane (99%), ethanol (99%), chloroform (99%), and acetone (99%) were purchased from Samchun Chemicals. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (mPEG-2000 PE) were purchased from Avanti Polar Lipids, Inc.

Synthesis of PEG-phospholipid-capped MnO nanoparticles (MnO@PEG-phospholipid). MnO nanoparticles, dispersed in chloroform, were encapsulated with a PEG-phospholipid shell. Typically, 2 ml of the organic, dispersible MnO nanoparticles in CHCl₃ (5 mg/ml) was mixed with 1 ml of CHCl₃ solution containing 10 mg of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] at a ratio of 5:1. After evaporating the solvent, it was incubated at 80 °C in vacuum for 1 h. The addition of 5 ml water resulted in a clear and dark-brown suspension. After filtration, excess mPEG-2000 PE was removed by ultracentrifugation, and the purified PEG-phospholipid capped MnO nanoparticles were dispersed in distilled water.

Synthesis of dense silica-coated MnO nanoparticles (MnO@dSiO₂). Dense silica-coated MnO nanoparticles were prepared as described previously along with some modifications. Polyoxyethylene(5) nonylphenyl ether (0.23 g, 0.544 mmol, Igepal CO-520, containing 50 mol% hydrophilic group, Aldrich) was dispersed in a scintillation tube, containing 4.5 mL of cyclohexane, by sonication. Next, 300 μl of MnO nanoparticles, dispersed in cyclohexane (10.8 mg Mn/mL), was added to the scintillation
tube. The resulting mixture was vortexed until the mixture became transparent. Next, 50 μl of ammonium hydroxide was added to form a transparent, brown reverse microemulsion. After 1h of stirring at room temperature, 50 μL of tetraethylorthosilicate (TEOS) was added. The reaction was continued for 24 h at room temperature. The resulting MnO@dSiO₂ particles were precipitated by adding ethanol, and collected by centrifugation. The collected nanoparticles were redispersed in ethanol, and purified by sonication and centrifugation. Washing steps were repeated three more times, and finally, the particles were redispersed in distilled water.

**Characterization of HMnO@mSiO₂ Nanoparticles.** Transmission electron microscopy (TEM) analysis was conducted with a JEOL JEM-2010 transmission electron microscope operating at 200 kV. Samples were prepared by putting a drop of particle dispersions onto a carbon-coated copper grid. X-ray diffraction (XRD) patterns were obtained using a Rigaku D/Max-3C diffractometer, equipped with a rotation anode and a Cu Kα radiation source (λ = 0.15418 nm). The phase identification was performed using JCPDS-ICDD 2000 software (The International Centre for Diffraction Data; ICDD). N₂ absorption isotherms were measured at 77 K using a Micromeritics ASAP 2000 gas adsorption analyzer after the samples were degassed at 293 K at 10 μtorr for 5 hours. Surface area and total pore volume was evaluated using the Brunauer-Emmet-Teller (BET) model, and the pore size was evaluated using the Barett-Joyner-Halenda (BJH) model. DLS measurements and zeta potential measurements were obtained using a zeta-potential and particle size analyzer (ELS-Z2, Otsuka). An inductively coupled plasma atomic emission spectrometer (ICP-AES, Shimadzu ICPS-1000IV-JAPAN) and an inductively coupled plasma mass spectrometer (ICP-MS, ELAN 6100, Perkin-Elmer SCIEX) were used for the quantitative analysis.

**Cell Culture.** Inguinal fat pads were isolated from male C57Bl/6J mice, washed in PBS, and blood vessels were dissected out. The fat was then minced and digested with 0.1% collagenase A (Roche) in HBSS++ (Gibco) for 30 minutes in a 35 mm dish at 37 °C and 5% CO₂ humidity. The cell suspension was then neutralized with MSC growth media, i.e., Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen), supplemented with 20% fetal bovine serum (FBS, Gibco) and penicillin/streptomycin (100 U/mL and 100 μg/mL, respectively, Gibco), and centrifuged at 1200 g for 10 minutes. The top layer of lysed, mature adipocytes, along with the supernatant, was decanted. The pellet, or the stromal vascular fraction, was resuspended in growth media and titrated before centrifuging at 1200 g for 5 minutes. The pellet was then resuspended with fresh MSC growth media and plated in a 35 mm dish until 100% confluence. MSCs were then passaged and expanded.
**Measurement of Intracellular Manganese Content.** After *in vitro* gelatin phantom imaging, samples were assayed for manganese content using inductively coupled plasma mass spectroscopy (ICP-MS) analysis (ELAN 6100; Perkin-Elmer SCIEX). The cellular manganese concentration was calculated by dividing the total manganese content by the number of cells.

**Fluorescence Microscopy.** To observe cellular uptake of nanoparticles, labeled MSCs were cultured in a six-well plate (Falcon #303046, Non-pyrogenic). After 24 h, cells were washed with PBS, fixed with 4% paraformaldehyde, and stained with Hoechst 33342 (0.1 μg/mL in PBS, Aldrich). Fluorescence images were acquired using Olympus BX51 and IX71 epifluorescence microscopes equipped with an Olympus DP-70 digital acquisition system.

**Cell Viability Assay.** To examine cell viability, an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (Cell Titer 96 Aqueous, G3582; Promega) was used. After labeling by electroporation or incubation, labeled MSCs were initially seeded in triplicate on 96-well plates at a density of 5x10^3 cells/well, and incubated in growth medium. After 24 h, cells were assayed for viability. To this end, Cell Titer 96® Aqueous One Solution reagent was added to each well. Cells were incubated for 2 h at 37 °C in a humidified 5% CO₂ atmosphere. The absorbance at 490 nm was measured using a 96-well plate reader (Beckman Coulter).

**In Vitro Cell Differentiation Studies.** Adipocyte differentiation was induced with an adipogenic differentiation kit (R&D System). HMnO@mSiO₂-labeled MSCs were washed with PBS, trypsinized, and plated at 2 × 10^4 cells/cm^2 in multiple wells of a 24-well plate in StemX Vivo adipogenic base medium (CCM007) or in regular growth medium as negative differentiation control. When cells in regular growth medium reached 100% confluence, they were fixed with 4% paraformaldehyde, stained with 0.5% Oil Red O (Sigma) in isopropanol. When cells in adipogenic base medium reached 100% confluence, the medium was gently aspirated and fresh adipogenic base medium with 1:100 adipogenic supplement (CCM011) was added to the wells. This complete adipogenic differentiation medium was changed every 3-4 days for 2.5 weeks. Cells were then fixed with 4% paraformaldehyde and stained with 0.5% Oil Red O (Sigma) in isopropanol. MSCs not labeled with HMnO@mSiO₂ underwent the same differentiation protocol, were stained, and served as positive differentiation control. Images were taken at 20X on phase light settings with an inverted light source microscope.

To induce osteocytic differentiation, an osteocytic differentiation kit was used (R&D Systems). HMnO@mSiO₂-labeled MSCs were washed with PBS, trypsinized, and
plated at $4 \times 10^3$ cells/300ul/well in multiple wells of a 24-well plate in StemX Vivo osteogenic base medium (CCM007) or regular growth medium (undifferentiated negative control). When cells in the regular growth medium reached 50-70% confluence, they were fixed in 4% paraformaldehyde, and stained with Alizarin red in distilled water at pH = 4.1-4.3. When the cells in the osteogenic base medium reached 50-70% confluency, the culture media was replaced with fresh osteogenic base medium containing StemXVivo Osteogenic Supplement (CCM008). This was changed every 3-4 days for 2.5 weeks. Cells were then fixed with 4% paraformaldehyde and stained with Alizarin red in distilled water at pH = 4.1-4.3. MSCs not labeled with HMnO@mSiO$_2$ underwent the same differentiation protocol, were stained, and served as positive differentiation control. Images were taken at 20X on phase light settings with an inverted light source microscope.
Figure S1. N₂ adsorption/desorption isotherms of H₉MnO@mSiO₂ (inset: pore size distribution; V: pore volume, D: pore size).

Figure S2. TEM images of (a) PEG-phospholipid-capped MnO nanoparticles (MnO@PEG-phospholipid, 15 nm MnO core, 35 nm total particle diameter). (b) dense silica-coated MnO nanoparticles (MnO@dSiO₂, 15 nm MnO core, 35 nm total particle diameter).
**Figure S3.** XRD pattern for HMnO@mSiO₂ nanoparticles before (a) and after (b) acid etching. XRD patterns for the tetragonal Mn₃O₄ phase (JCPDS Card No. 24-0734) and cubic MnO phase (JCPDS Card No. 07-0230) are shown for reference.

**Figure S4.** Hydrodynamic size distribution of HMnO@mSiO₂ nanoparticles in PBS, as measured by dynamic light scattering (DLS). The average hydrodynamic diameter size was 86 nm.
**Figure S5.** Electrophoretic (zeta) potential ($\zeta$) was measured with particle size and a zeta potential analyzer. The zeta potential of HMnO@mSiO$_2$ nanoparticles in PBS solution was -30.43 (mV).

**Figure S6.** Assessment of MSC viability at 24 h after electroporation or simple incubation with HMnO@mSiO$_2$ nanoparticles. Cell viability was examined using an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay.
**Figure S7.** Differentiation of HMnO@mSiO$_2$-labeled MSCs into adipogenic (a-d) or osteogenic (e-h) phenotype. The following labeling conditions were used: (a and e) MSCs electroporated with HMnO@mSiO$_2$; (b and f) MSCs electroporated without HMnO@mSiO$_2$; (c and g) MSCs labeled with HMnO@mSiO$_2$ but not electroporated; and (d and h) MSCs without electroporation or HMnO@mSiO$_2$.

**Figure S8.** Assessment of the viability of human breast adenocarcinoma cells (MCF-7 cells) with HMnO@mSiO$_2$ nanoparticles. Cell viability was examined using an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay.
Figure S9. Measured relaxivities of additional preparation of HMnO@mSiO₂ nanoparticles at 11.7 T. (a) Plot of 1/T₁ versus Mn concentration. The slope indicates the specific relaxivity (r₁). (b) Plot of 1/T₂ versus Mn concentration. The slope indicates the specific relaxivity (r₂).

References for Supporting Information

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