Hollow Structured Mesoporous Silica Coated MnO Nanoparticles as Highly Efficient T1 Contrast Agents and Their Applications in MR Tracking of Transplanted Mesenchymal Stem Cells

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Introduction

Accurately tracking the migratory fate of the transplanted cells continues to be a major challenge in stem cell research. This challenge could potentially be overcome by labeling the transplanted cells with magnetic nanoparticles (np) and tracking them with magnetic resonance imaging (MRI). In the last decade, superparamagnetic iron oxide (SPIO) np has become the gold standard for MRI cell tracking (1-3), and has even entered clinical use (4). However, in many cases, SPIO-labeled cells cannot be distinguished from other hypointense regions on T2/T2*-weighted MR images. An alternative MR contrast agent is manganese oxide (MnO) np, which can influence tissue contrast both via T1 and T2 contrast mechanism. Previously, MnO nps coated with poly-ethylene-glycol (PEG) (5) were used to demonstrate feasibility of cell labeling and *in vivo* MRI tracking (6). In this study, we report a novel design of MnO nps, which have 'hollow' structures in mesoporous silica coating on the surface (termed HMnO@MSN). Their enormous surface area to volume ratio increases water accessibility to the manganese core and provides an efficient T1 contrast MR imaging. We labeled mesenchymal stem cells (MSCs) with HMnO@MSN and injected them into the mouse brain for visualization by MRI.

Methods

MnO np coated with mesoporous silica shells were prepared by silica sol-gel reaction using CTAB (cetyltrimethylammonium bromide) as the organic template to make mesopores followed by etching with hydrochloric acid (7). Transmission electron microscope (TEM) was used to characterize synthesized nanoparticles.

Cellular imaging *in vitro*: 2 million MSCs suspended in 580 µl PBS were mixed with 120 µl HMnO@MSN (or PBS as control) and electroporated or incubated as described previously (8). To evaluate the intracellular uptake of nps, rhodamineisothiocyanate was used to label HMnO@MSN, which was visualized by fluorescence microscopy. MRI scans for *in vitro* phantoms were taken using a Bruker 9.4T MRI scanner. T1 relaxation was measured using MSME pulse sequence (for T1; TE= 9 ms, and TRs= 10, 6, 4, 2, 1.5, 1.0, 0.8, 0.5, 0.3, 0.2 s). All data processing was performed using MATLAB (Mathworks, Natick, MA).

Cellular imaging *in vivo*: 1.0×10^5 MSCs labeled with nps or unlabeled were injected into the striatum of mice (n=2 respectively). Serial MRI scans were performed thereafter using a Bruker 9.4T horizontal bore magnet equipped with a 30-mm Sawtooth resonator (Bruker) using a MSME pulse sequence. For T1 measurement, the following parameters were used: TE= 10.5ms, TRs= 6, 4, 3, 2, 1.5, 1.0, 0.8, 0.5, 0.3s, FOV= 2.1×2.1cm and matrix size was 256×96 for the anatomical images.

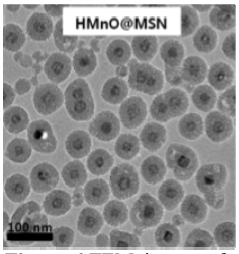

Figure 1. TEM image of HMnO@MSN

Results

HMnO@MSN (core size ~ 15nm, overall size ~ 60nm) were successfully synthesized and well-dispersed in water and PBS. TEM images clearly show the hollow-structured, etched cores and mesoporous structure of shells (**Fig. 1**). These mesoporous structures enable water molecules to be easily accessible to the magnetic core while providing a large water-accessible surface area. **Fig. 2a** demonstrates that the tube containing MSCs electroporated with the highest nps concentration (i, 200 µg Mn/ml) is much brighter on T1-weighted images due to shortening of the MRI spin-lattice relaxation time (T1). Electroporated cells contain more particles resulting in shorter T1 relaxation than others (**Fig. 2b**). **Fig. 2c** shows linear dependency of R1 on the nps concentration that was used for labeling. The cellular uptake of the nps was also verified by nps conjugated to fluorescent dye (RITC). For the same nps concentration, MSCs labeled by electroporation showed more fluorescence verifying higher uptake of nps.

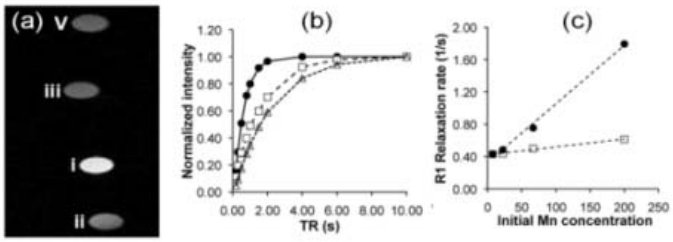

Figure 2. T1 weighted MRI image of tubes containing cells suspended in 5% gelatin. (a) The cells in each tube were electroporated with different initial nps concentrations, (i-v; 200, 66.67, 22.22, and 0 µg Mn/ml respectively). (b). The MRI signal intensity of cells that were treated with nps [200 µg Mn/ml] using electroportation (●) incubation (□) or electroporation without nps (△). (c) The R1 relaxation time plotted as a function of the nps concentration used for labeling the cells (electroportation (●); incubation (□)).

Fig. 3 shows the result of *in vivo* MRI imaging of MSCs electroporated with HMnO@MSN. No hyperintense signal was detected when unlabeled MSCs were transplanted (**Fig. 3a, red arrow**). On the contrary, hyperintense signal appeared and remained in MRI for 14 days post injection of MSCs labeled with HMnO@MSN (**Fig. 3b, green arrows**).

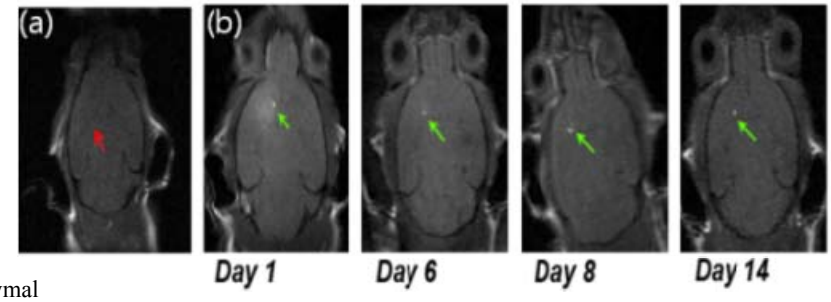

Figure 3. *In vivo* MRI of transplanted MSCs labeled with HMnO@MSN at 9.4T MR scanner

Conclusions

Novel, hollow structured mesoporous silica-coated MnO nps (HMnO@MSN) were successfully synthesized and showed significantly high T1 and T2 contrast. Mesenchymal stem cells were effectively labeled with HMnO@MSN with the use of electroporation, and following intracranial injection, could be clearly visualized as a hyperintense signal for at least 14 days.

References (1) J. W. Bulte, D. L. Kraitchman, NMR Biomed 17, 484 (Nov, 2004). (2) M. S. Thu et al., PLoS One 4, e7218 (2009). (3) A. S. Arbab et al., Stem Cells 24, 671 (Mar, 2006). (4) I. J. de Vries et al., Nat Biotechnol 23, 1407 (Nov, 2005). (5) H. B. Na et al., Angewandte Chemie International ed. 46, 5397 (2007). (6) A. A. Gilad et al., Magn Reson Med 60, 1 (Jun 25, 2008). (7) J. Kim et al., Angewandte Chemie International ed. 47, 8438 (2008). (8) P. Walczak et al., Magn Reson Med 54, 769 (2005).