Small and ultra-small nanoparticles of manganese oxide (SPMnO, USPMnO) for positive contrast in cellular MRI

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Background: Manganese oxide nanoparticles (MnO) made of ultra-small nanocrystals are used to label and visualize implanted cells *in vivo,* in pre-clinical studies.*[1, 2]* They are being developed as a complement to superparamagnetic small and ultra-small iron oxide nanoparticles (SPIO and USPIO). Such contrast agents have a more significant impact on the spin-lattice relaxation time (T_1) than on T_2. MnO-labeled cells appear bright in T_1-weighted MR images, leading to finer delineation of local accumulations of these cells compared with procedures using iron oxide contrast agents coupled with T_2 and T_2^*-weighted imaging sequences.

Introduction: Nanoparticle size, agglomeration behavior and surface chemistry are of utmost importance in cell labeling efficiency, and these aspects have not been thoroughly investigated with MnO particles. First, agglomeration is expected to impact on T_2, affecting therefore the capacity of the nanoparticulate contrast agent to perform as a "positive label".*[3]* In one form or another, agglomeration occurs when particles are internalized in the cells; therefore, it is necessary to study *in vitro* the relaxometric performance of MnO aqueous nanoparticle suspensions in both agglomerated (SPMnO) and individual (USPMnO) forms, using an appropriate nanoparticulate model. Based on previously established data for iron oxide particles, agglomerates (25-150 nm) should be ingested more efficiently than individual ultra-small nanoparticles (~5-25 nm). As a next step in the development of SPMnO and USPMnO, it is necessary to compare the Mn ingestion and MR signal-enhancement efficiency of MnO agglomerates (SPMnO) internalized in cells, and compare this signal-enhancement modulation with that offered by USPMnO-labelled cells. Despite strong cytotoxicity limitations, Mn^{2+} ions are used in manganese-enhanced imaging procedures (MEMRI), and the performance of MnO nanoparticles as cell labels must be systematically compared with this contrast agent. Finally, one of the major advantages sought in the development of "T_1-weighted" cellular MRI procedures, is the potential capacity to correlate signal, volume, the amount of locally accumulated cells. This last step requires the development of appropriate MR signal quantification tools to correlate signal-enhancement effects and cell pellet volumes in MR images acquired in T_1-weighted sequences.

Materials and methods: *MnO nanoparticles* showing a diameter of oxide cores ~ 8 nm (Figure 1.a), were synthesized using a thermal decomposition technique.*[1]* The particles were then coated with a thiol-containing ligand (dimercaptosuccinic acid, DMSA), with addition of polyethylene glycol (PEG) to enhance steric repulsion. This is the first study to report on the use of a thiolated coating to increase manganese nanoparticles cell labeling efficiency. DMSA as a surface ligand also allows the fabrication of both stable "agglomerates" (~75-105 nm ∅) and "individual nanoparticles" (~12-25 nm ∅; Figure 1.b), by proceeding to a strong alkaline treatment prior to use in physiological conditions.*[3]* After synthesis, the particles were dialyzed, imaged with transmission electron microscopy (TEM) to determine the core diameter and dynamic light scattering (Malvern Nanosizer 173) to establish their total hydrodynamic size distribution. Then, T_1 and T_2 of the SPMnO and USPMnO aqueous suspensions were measured at 60 MHz (Bruker Minispec) and at varying external magnetic field by NMRD (nuclear magnetic relaxation dispersion, Stelar). In this comparative study, USPMnO, SPMnO and $MnCl_2$ were used to label HT-1080 (human fibrosarcoma) cells, at increasing Mn concentrations for 2h incubation. After incubation, the cells were thoroughly rinsed, harvested, washed, measured for viability, and distributed in centrifugation tubes for MR visualization (1.0T; 25ºC; 5.5 cm ∅ rat coil; T_1-w. spin-echo 2D: TE/TR: 9.6ms/718ms; FOV: 60 mm; 1.0 mm contiguous slices, 400x400; 3 NEX). The total scan time was 17 min., and the final voxel resolution was estimated to 150µm x 150µm x 1mm. MR images of signal-enhanced cell pellets were analyzed using an algorithm developed for the public-domain ImageJ software, which allowed the precise quantification of the total amount of signal generated in each cell pellet. In order to correlate signal intensity and Mn concentration, all paramagnetic suspensions and labeled cell pellets were carefully digested and then precisely measured with GF-AAS.

Results: Agglomerated and individual nanoparticles (SPMnO and USPMnO, figure 1) were used in this study, both coated with DMSA and PEG. The highest r_1 relaxivities were achieved with SPMnO nanoparticles (r_1 = 7.48 $mM^{-1}s^{-1}$; r_2/r_1 = 4.5), compared with USPMnO (r_1 = 3.00 $mM^{-1}s^{-1}$; r_2/r_1 = 3.63), as demonstrated by both static field and NMRD results (Figure 2). *In vitro,* USPMnO presented relaxometric parameters slightly more optimal for T_1-weighted imaging. Our results also demonstrate that thiolated nanoparticles (DMSA-PEG coatings) are efficiently internalised in cells. The nanoparticle-labeled cells are viable after a 2-h incubation treatment with high concentrations of Mn in the incubation medium (3.5 mM); the same concentration of Mn added in the form of Mn^{2+} ions ($MnCl_2$) dramatically affects cell viability. Therefore, nanoparticles appear much less cytotoxic than Mn^{2+} ions, allowing meanwhile a much faster manganese cell uptake (Figure 4). SPMnO are internalised at least twice as fast as USPMnO (Figure 5). The total signal intensity per cell, normalised to the amount of internalised Mn, did not indicate a significant difference in contrast enhancement between SPMnO and USPMnO. The T_1-weighted imaging protocol and the labelling methodology used in this study, allowed the clear visualization (Figure 3) and quantification (Figures 4,5) of cell pellets down to ~10000. This is a significant achievement for a positive contrast agent.

Conclusion: Agglomerates of ultra-small manganese oxide nanoparticles (SPMnO) internalise at least twice as fast as individual nanoparticles (USPMnO). Coating the particles with DMSA enhance the nanoparticle internalisation process, while preserving the cell viability. Cells labelled with MnO appear bright in T_1-weighted MR images. Although internalization is more efficient with nanoagglomerates (SPMnO), when normalised for cellular Mn content, both contrast agents provide the same signal-enhancement effect, which is in accordance with their similar r_2/r_1 ratio. The signal intensity achieved per cell, normalized to the Mn contents per cell, indicates a plateau. The strong accumulation of Mn per cell raises the possibility of maintaining an efficient positive contrast effect in cells over several cell duplication cycles.

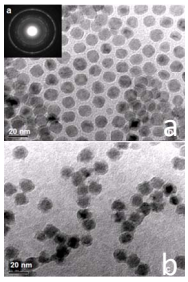

Figure 1: TEM (120 keV)

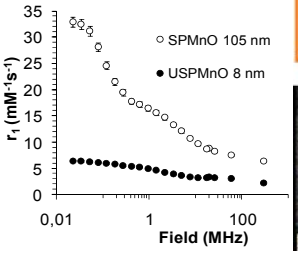

Figure 2: NMRD

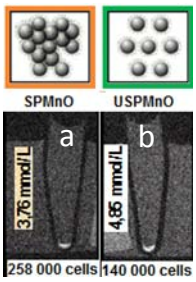

Figure 3: MRI 1T

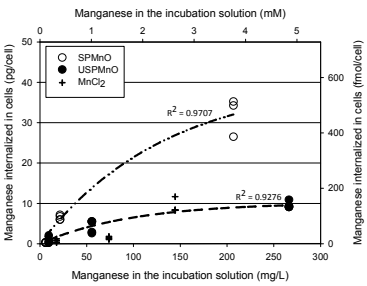

Figure 4: Mn per cell

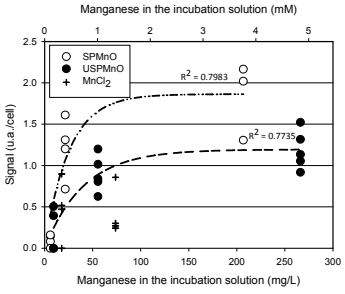

Figure 5: Signal per internalised Mn

[1] H. B. Na, et al., *Angewandte Chemie Int. Ed.* **2007**, *46*, 5397-5401. [2] A. A. Gilad, et al., *Magn Reson Med* **2008**, *60*, 1-7. [3] A. Roch, et al., *Journal of Magnetism and Magnetic Materials* **2005**, *293*, 532-539. [4] N. Fauconnier, et al., *J Colloid Interface Sci* **1997**, *194*, 427-33. [5] A. Roch, et al., *Journal of Magnetism and Magnetic Materials* **1999**, *201*, 77-79.