Magnetic Resonance Imaging of Stem Cells Labeled with Micrometer-sized Iron Oxide Particles: Applications to Musculoskeletal Tissue Engineering

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Introduction: Adult stem cells are multi-potent cells in the body that function as a source of undifferentiated cells for the rejuvenation of various tissues. In conditions where the body is unable to repair or regenerate tissue on its own, isolating these cells from patients, culturing them *ex vivo*, and transplanting them back into the body has shown great promise. In particular, mesenchymal stem cells (MSCs), a subset of adult stem cells, have demonstrated the ability to differentiate into a variety of cell types including, chondrocytes (cartilage), osteocytes (bone), and myocytes (muscle) [1]. To aid in the development and implementation of clinically viable stem cell-based tissue engineering therapies, a technique is needed to monitor implanted cells throughout the course of treatment. Labeling of MSCs with an iron oxide contrast agent prior to implantation has the potential to allow for longitudinal non-invasive *in vivo* assessment of the bio-distribution of transplanted cells via magnetic resonance imaging (MRI) [2]. Micrometer-sized iron oxide particles (MPIOs), a type of superparamagnetic iron oxide (SPIO), have demonstrated effective labeling of stem cells for MR tracking [3]. These particles consist of an iron oxide magnetite core encased within a divinyl benzene inert shell, along with a fluorescent dye for optional microscopy co-localization. The size of a single particle (~ 1 μm) is approximately three orders of magnitude larger than conventional SPIO nano-particles, which enables MR detection of cells labeled with only a few particles [3]. Studies by Hinds *et al*. [3] have demonstrated efficient non-toxic labeling of MSCs, longitudinal MR imaging of labeled cells, and the ability of labeled MSCs to undergo adipogenesis and osteogenesis *in vitro*. This study aims to further investigate MPIO labeling, and its applications in longitudinal monitoring of stem cell-based musculoskeletal tissue engineering.

Methods: For all studies, primary cultures of rabbit MSCs (rMSCs) were used (passage 4-8). Cells were labeled with commercially available 1.63 μm diameter MPIOs (Bangs Laboratories, Fishers, IN) by adding the iron oxide suspension (10 μL/mL) to the growth medium and incubating with the cells for 18 hours [3]. Following labeling, excess iron oxide was removed by multiple washes with phosphate buffered saline (PBS) [3]. Cells were then either trypsinized and collected for MRI (initial detection), or maintained under normal culture conditions (including passaging at confluency) for longitudinal MRI at 1, 3, and 6 weeks post-labeling. For MRI, cells ($5x10^4$-$5x10^5$ cells/mL) were seeded in Puramatrix hydrogel (3DM, Cambridge, MA) according to the manufacturer's instructions in 10 mm nuclear magnetic resonance (NMR) tubes (n = 2 per time point) with a total volume of 1 mL in each tube. High-resolution MR imaging was performed on a 600 MHz scanner (Varian, Palo Alto, CA) using a 10 mm broadband coil (Varian). Imaging experiments were repeated in duplicate, and included unlabeled rMSC as well as empty Puramatrix hydrogel as controls. Gradient echo multi-slice (GEMS) 2D imaging was performed using a repeat time (TR)/echo time (TE) = 200/4.48 ms with a scan time of ~7 min. Spin echo multi-slice (SEMS) 2D imaging was performed using a TR/TE = 1000/15 ms, with a scan time of ~17 minutes. Both sequences were acquired with a slice thickness = 0.4 mm, field of view (FOV) = 10 mm, and matrix = 256x256, resulting in an in-plane resolution of 40 μm. Obtained images were cropped to a sub-region of the entire tube, and line profile analysis of the obtained images was performed using VnmrJ software (version 2.3A, Varian) and Microsoft Excel.

Results: High-resolution MRI of labeled cells encapsulated within Puramatrix hydrogels demonstrates initial detection using both GEMS and SEMS sequences. Figure 1a,b shows labeled and unlabeled cells respectively imaged as a single slice at a concentration of $5x10^4$ cells/mL with a GEMS sequence. The susceptibility artifact and resulting signal void caused by the presence of MPIOs appears strongest with gradient echo (GRE) imaging. Fig 1c,d shows the same slice imaged with a SEMS sequence and demonstrates similar detection of labeled cells using spin echo (SE) imaging. Line profile analysis of 10 points corresponding to signal voids in Figure 1a, and Figure 1c are shown in Figure 2a and Figure 2b respectively. While the presence of iron oxide causes a similar drop in signal intensity (40% of the background gel intensity) at the center using both sequences, the effect of the MPIO particles as a function of the distance from the void center appears to be greater for the GEMS sequence. Longitudinal imaging of the labeled cells encapsulated within Puramatrix hydrogel demonstrates detection of labeled cells using both sequences at 1 and 3 weeks post-labeling (Fig. 3). There also appears to be a progressive decrease in the number of cells detected between the initial, 1 week, and 3 week time points.

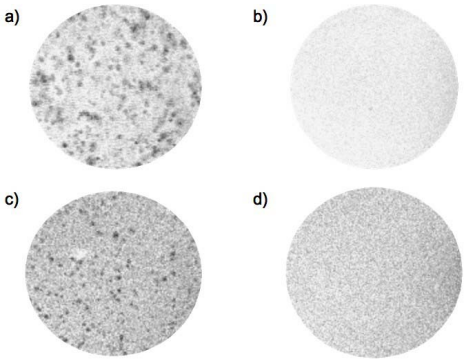

Figure 1: High-resolution single slice MRI of hydrogel encapsulated MPIO-labeled rMSCs: GEMS imaging (TR/TE = 200/4.48 ms) of (a) MPIO-labeled cells; and (b) unlabeled rMSC. SEMS imaging (TR/TE = 1000/15 ms) of (c) MPIO-labeled cells; and (d) unlabeled rMSC. Both sequences demonstrate signal loss associated with labeling. (cell conc. = $5x10^4$ cells/mL; resolution = 40 μm)

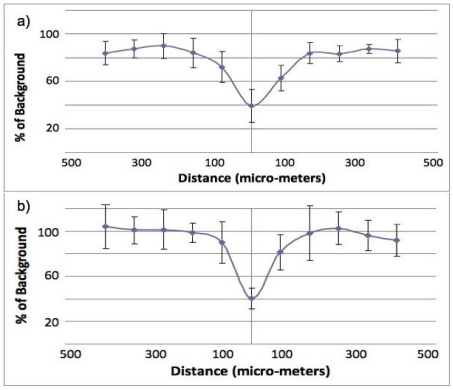

Figure 2: Line profile analysis of high-resolution MR single slice image: n = 10 hypo-intense signal points were chosen and the signal intensities (as a % of the background gel) measured as a function of horizontal distance from the chosen point. (a) GRE image; (b) SE image. (cell conc. = $5x10^4$ cells/mL; error bars represent ± standard deviation)

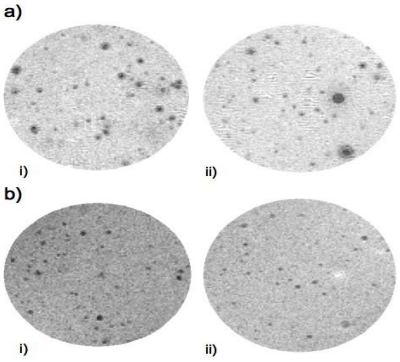

Figure 3: Longitudinal high-resolution MRI of labeled hydrogel encapsulated cells: (a) single slice GEMS image (TR/TE = 200/4.48 ms) at (i) 1 week; and (ii) 3 weeks following labeling. (b) single slice SEMS image (TR/TE = 1000/15 ms) at (i) 1 week; and (ii) 3 weeks following labeling. (cell conc. = $5x10^4$ cells/mL)

Discussion: This study demonstrates the promise of MPIO labeling and subsequent MRI as a technique to visualize and longitudinally monitor implanted stem cells. In particular, these results indicate the ability to detect labeled cells encapsulated within a commercially available hydrogel with near cellular resolution. While the GEMS sequence is more sensitive to the presence of iron oxide than the SEMS sequence, it is also more sensitive to the presence of air bubbles, which can form during the gelation process of the Puramatrix. Consequently this highlights a tradeoff between the increased sensitivity of the GEMS sequences, and the potentially higher accuracy of detecting only labeled cells with the SEMS sequence. Current and future studies are aimed at using confocal microscopy to verify the presence of MPIO particles within rMSCs following labeling, as well as detection of labeled cells within a mouse model of muscle tissue degeneration. These results together with future studies are expected to aid in the development and implementation of stem cell-based musculoskeletal tissue engineering strategies.

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