Iron Oxide Labeling of Mesenchymal Stem Cells with Micrometer-sized Particles: Applications to Cartilage Tissue Engineering

K. Saldanha[1], R. Doan[1,2], and S. Majumdar[1]

[1]Department of Radiology, UCSF, San Francisco, CA, United States, [2]Cleveland Clinic Lerner College of Medicine, Cleveland, OH, United States

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 chondrocytes [1], with applications to the treatment of focal articular cartilage defects or osteoarthritis (OA). 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 oxides (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 (~ 1μm) 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]; labeling with SPIO nano-particles often requires millions of particles per cell to enable MR detection. 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 determine the effect of labeling on *in vitro* chondrogenesis of MSCs, which has not been examined to date.

Methods

Primary cultures of rabbit MSCs (rMSCs) were labeled with commercially available 1.63μm diameter MPIOs (Bangs Laboratories, Fishers, IN) by adding the iron oxide suspension (10μL/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], and the cells collected. MR imaging was performed on a 3 Tesla scanner (GE Medical Systems, Waukesha, WI) using an 8-channel transmit/receive phased array knee coil (GE). For *in vitro* experiments, rMSCs at a concentration of $1x10^6$ cells/mL were suspended in tubes containing Ficoll (Amersham Biosciences; Piscataway, NJ) to prevent cell settling. Imaging experiments were repeated in triplicate, and included unlabeled rMSC ($1x10^6$ cells/mL) as well as empty Ficoll controls. The tubes were placed in a plastic container filled with water to remove the air susceptibility artifact and imaged coronally as a single slice at room temperature (20°C). T_2*-weighted imaging was performed using a gradient echo (GRE) sequence with TR=500ms, and TE=4,6,8,12,20, & 24ms. T_2-weighted imaging was performed using a spin echo (SE) sequence with TR=2000ms, and TE=20,40,50,60,80,100,150, & 200ms. Both sequences resulted in a resolution of .47 x .47 x 3 mm^3, and FOV = 12cm. Images were analyzed using software developed in Interactive Data Language (Research Systems, Boulder, CO).

Chondrogenesis via pellet culture was induced using a Chondro-Bulletkit containing TGF-β3 (Lonza; Walkersville, MD). Labeled rMSCs and unlabeled rMSCs were differentiated according to the manufacturer's protocol (4 weeks). Following differentiation, labeled pellets (n=6) and unlabeled pellets (n=3) were fixed in 10% formalin, and paraffin embedded according to standard techniques. Embedded sections (5μm) were stained with hematoxylin and eosin (H&E), and safrinin-O/fast green, visualized by light microscopy (Olympus CX41; Center Valley, PA), and photographed (Nikon Coolpix 5000; Melville, NY). Glycosaminoglycan (GAG) content was determined by measuring chondroitin sulfate concentration using dimethylmethylene blue dye (DMB) (Biocolor; Carrickfergus, UK), and differences between labeled (n=6) and unlabeled cells (n=6) compared using a student's t-test ($p < .05$). Fluorescence and bright field microscopy of rMSCs was performed using an Olympus BX60 microscope (ex/em: 480/520nm), and photographed (Zeiss Axiocam MRm; Maple Grove, MN). NIH ImageJ was used for image processing.

Results

Incubation of the contrast agent with rMSCs resulted in cellular uptake of the MPIOs, which can be detected by both fluorescence microscopy, and MR imaging. Figure 1a shows a fluorescence image of a single rMSC following the overnight labeling procedure and subsequent PBS washing. The fluorescent tag component of the MPIO particles demonstrates localization within cell, with multiple regions containing contrast agent (Fig. 1a). An unlabeled cell is shown for comparison (Fig. 1b). GRE and SE imaging both demonstrate the strong signal loss associated with labeled cells (Fig. 1c,f), which is not present with unlabeled cells (Fig. 1d,g) or empty Ficoll alone (Fig. 1e,h). Differentiation studies demonstrated positive indicators of chondrogenesis in both labeled and unlabeled pellets (Fig. 2). While labeled sections show traces of positive GAG staining (red), the presence of large amounts of extracellular iron hinders visualization of the extracellular matrix (ECM) (Fig. 2b). However, the results of the GAG assay confirm the presence of GAGs, with no significant difference between labeled and unlabeled cells (Fig. 2c).

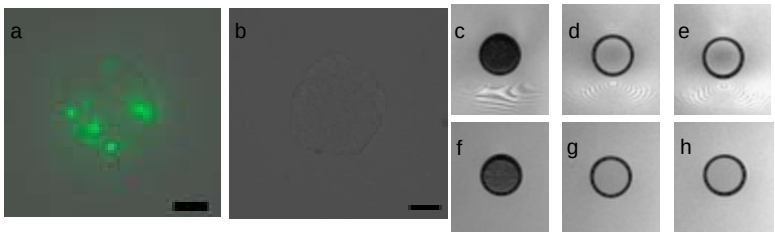

Figure 1: MPIO labeling: (a) fluorescence microscopy of (a) labeled rMSC; (b) unlabeled rMSC; (c) (d) & (e) GRE image (TE/TR: 8/500ms) of labeled cells, unlabeled cells, and Ficoll respectively; (f) (g) & (h) SE image (TE/TR: 100/2000ms) of labeled cells, unlabeled cells, and Ficoll respectively. (cell conc. = $1x10^6$ cells/mL; scale bar = 10 μm)

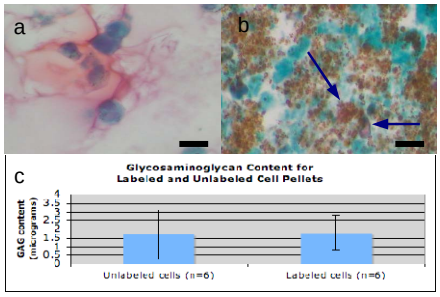

Figure 2: Chondrogenesis of labeled rMSCs: staining of (a) unlabeled rMSCs; and (b) labeled rMSCs. GAG stains red, nuclei black, and the cytoplasm green. Labeled cells show positive GAG staining (arrows), as well as extracellular iron; (c) results of GAG assay show no significant difference between labeled and unlabeled cells. (scale bar = 20 μm)

Discussion

These studies demonstrate that MPIO labeling results in signal loss that can be visualized using both GRE and SE sequences at field strengths similar to that used clinically. The ability to track a population of labeled cells using a clinical scanner would be useful for monitoring tissue regeneration of articular cartilage. While labeling does not appear to inhibit chondrogenesis, stained sections show extracellular iron aggregates. This may suggest that PBS washing maybe be insufficient to remove excess iron following labeling, and the induction of pellet culture (specific to chondrogenesis) promotes the formation of these aggregates. Alternatively, if excess iron is in fact completely removed by PBS washing, the iron aggregates could be the result of cellular release during pellet formation and subsequent chondrogenesis. Regardless of the cause, this extracellular iron would interfere with the ability to track cells implanted for cartilage repair. Current and future studies are aimed at determining the source of this extracellular iron, and examining the use of 3D scaffolds to promote chondrogenesis of labeled cells without pellet culture.

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References:

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