In vivo MRI-Based Cell Tracking Using Bio-MPIOs

M. K. Nkansah[1], D. Granot[2], T. L. Lobo[2], and E. M. Shapiro[2]
[1]Department of Biomedical Engineering, Yale University, New Haven, CT, United States, [2]Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, CT, United States

INTRODUCTION: Previously, we described the fabrication of PLGA encapsulated iron oxide nano- and microparticles (Nkansah, et al, ISMRM 2010). These particles are important advancements for molecular and cellular MRI for a number of reasons. First, they incorporate large amounts of iron, especially relative to dextran coated (U)SPIO. Secondly, PLGA has favorable degradation characteristics, with particles degrading within a few weeks, and iron dissolution complete at 100 days. Thirdly, PLGA is an FDA approved material with a long history of use in drug delivery, enabling a clear trajectory towards potential clinical use. Here we describe design improvements to PLGA encapsulated iron oxide nano- and microparticles, magnetic cell labeling experiments, cell differentiation and cytokine release experiments, and ultimately, the first *in vivo* demonstration of MRI-based cell tracking using PLGA encapsulated iron oxide microparticles, or, Bio-MPIOs.

MATERIALS AND METHODS:

Magnetic particle design elements: Two different design elements were optimized in an effort to improve labeling efficiency and particle stability. First is the loading potential of PLGA encapsulated iron oxide nano- and microparticles. Here, nano- and microparticles were fabricated with increasing amounts of magnetite and assayed with ICP and SEM. Second, magnetic PLGA nanoparticles were formulated for cryoprotection using a variety of sugars.

Cell studies: Three experiments were performed to assay cell labeling and potential negative effects of internalized particles on cells. The first was to assay magnetic PLGA nano- and microparticles for cell labeling kinetics over 24 hours in culture. Separate dose and time dependent experiments were performed using mouse mesenchymal stem cells (mMSCs). Inert MPIOs were also assayed. Iron content at each time point was assayed using ICP. The viability of labeled cells was assayed using flow cytometry and compared to control particles containing only fluorescence, no magnetite. A second experiment investigated the ability of mMSCs to differentiate down adipogenic and osteogenic lineages following labeling with the same particles. Lastly, macrophages were labeled with magnetic PLGA nanoparticles and were assayed for cytokine release. Controls were unlabeled cells and cells labeled with inert MPIOs or control particles containing only fluorescence.

In vivo MRI: MRI-based cell tracking was tested using our well developed paradigm of *in vivo* labeling and tracking of endogenous rat neural progenitor cells (Shapiro, et al, NeuroImage 2006), using both inert MPIOs and Bio-MPIOs. Particles (20 µl of 10 mg/ml particles) were injected into the lateral ventricles of the brains of 6-week old rats (n=4 for each particle type) and rats underwent high resolution (100 microns isotropic) 3D gradient echo MRI at 11.7T over the course of two weeks. Neural progenitor cells phagocytose these particles at the ventricle and carry them as they migrate to the olfactory bulb, revealing dark contrast on T_2* weighted MRI.

RESULTS and DISCUSSION: It was determined that the maximal particle loading during fabrication was achieved using a feed ratio of 2:1 magnetite to PLGA, both for nanoparticles and microparticles. SEM images of these particles are shown in Figure 1. Blank microparticles were 1.3 ± 0.3 µm while heavily magnetic microparticles increased in size to 2.5 ±1.1 µm, likely due to viscosity changes. Magnetic nanoparticles showed much less size change versus blank nanoparticles. Cryoprotection is important to maintain nanoparticle dispersion following resuspension. For cryoprotection of magnetic PLGA nanoparticles, final lyophilization in 1% sucrose or 2% dextrose was sufficient to maintain monodispersity as assayed by DLS. Importantly, DLS revealed that nanoparticles which measured ~ 130 nm by SEM were sized at ~ 220 nm by DLS, due to the hydration shell that forms around the particles, revealing the hydrodynamic size the cell/animal/patient really interacts with.

For the cell labeling studies, Figure 2A shows that magnetic cell labeling occurred both in a time and dose dependent manner. Figure 2B shows a confocal microscopic image of a mMSC labeled with Bio-MPIOs. Viability studies using Sytox Blue dead cell stain revealed 92-95% viability for cells labeled with inert MPIOs, blank PLGA microparticles containing fluorescence only, or magnetic and fluorescent PLGA encapsulated iron oxide microparticles. Equivalent osteogenic and adipogenic potential was observed for cells labeled with both cryoprotected and non-cryoprotected nanoparticles as well as Bio-MPIOs and inert MPIOs, as revealed by staining samples either for bone matrix or intracellular fat globules (Figure 3B,C). Lastly, macrophages (derived from cytokine treated mouse bone marrow cells) labeled with either fluorescent only or dual fluorescent and magnetic PLGA nanoparticles (50% w/w magnetite) secreted high levels of TNF-α following stimulation with LPS (Fig 3A). This was the case with inert MPIOs as well. Importantly, magnetically labeled cells did NOT secrete TNF-α without stimulation, indicating that magnetic labeling itself does not stimulate cells. This is crucial for preserving the potential for using MRI to track macrophage homing to disease/ injury.

Figure 4 shows MRI slices from a rat injected with Bio-MPIOs (Fig 4A) and inert MPIOs (Fig 4B). As can be seen, injection of magnetic PLGA microparticles clearly enables the visualization of this migration pathway with as good, if not better fidelity than the Bangs MPIOs traditionally used for this. This is the first demonstration of MRI-based cell tracking using Bio-MPIOs and is encouraging for the development of these particles for clinical MRI-based cell tracking.

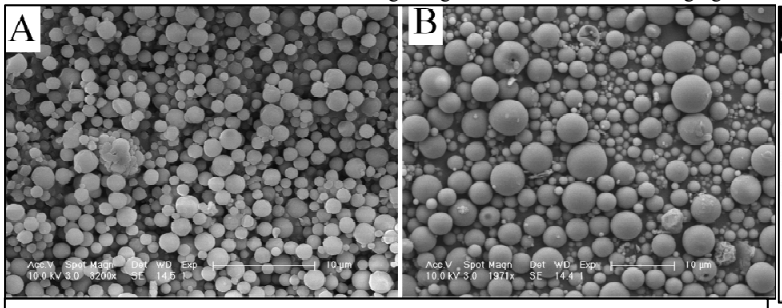

Figure 1: SEM of (A) empty and (B) magnetic Bio-MPIOs (2:1 magnetite:PLGA).

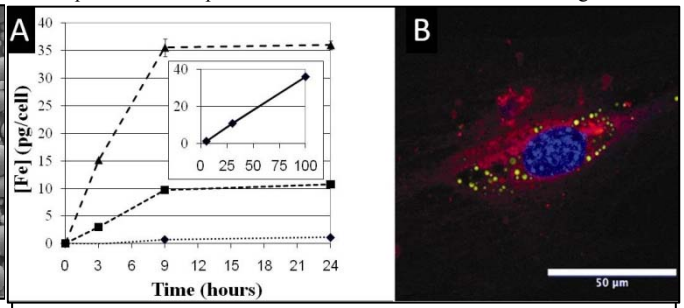

Figure 2: A) Cell labeling using Bio-MPIOs. Long dashed, short dashed and dotted lines are 100, 30 and 5 µg/ml Fe in labeling media, respectively. Inset is plot of [Fe µg/ml] in labeling media vs achieved pg Fe/cell. B) Confocal microscopy of Bio-MPIOs in a mMSC at 24 hours.

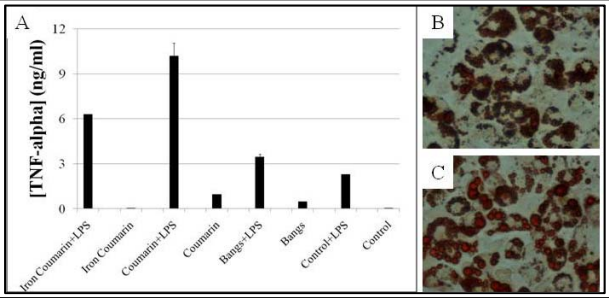

Figure 3: A) ELISA of TNF-α release following labeling for 16 hours. B) Unlabeled and (C) Bio-MPIO labeled mMSCs differentiated to adipocytes.

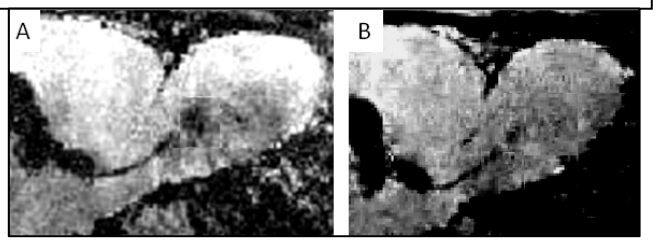

Figure 4: MRI-based cell tracking of endogenous neural progenitor cell migration using A) Bio-MPIOs and B) inert MPIOs. Magnetically labeled cells are detected as dark contrast moving from left to right.

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