Signal-Enhanced Visualization and Quantification of SPIO-Labeled Stem Cells

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Introduction: The noninvasive visualization and *in vivo* quantification of magnetically labeled cells has become an important area of development with the growing interest in cellular therapies. Superparamagnetic nanoparticles are useful contrast agents to label cells for visualization by MRI. Common strategies for imaging these labeled cells have included using standard fast spin echo and gradient echo sequences to observe the associated signal voids or negative contrast. However, signal voids may arise from other sources (e.g. absence of tissue, motion). Positive contrast mechanisms have been proposed (1,2) and promise improved contrast and specificity. We recently developed the *I*nversion *R*ecovery *On*-Resonant water suppression (IRON) method for MR imaging that results in positive signal enhancement of magnetically-labeled cells. In this study, we investigated the use of IRON for *in vitro* and *in vivo* detection and quantification of stem cells labeled with a superparamagnetic iron oxide (SPIO) contrast agent.

Methods: Cell preparations: Mesenchymal stem cells (MSCs) were isolated from bone marrow and culture-expanded for two passages as previously described (3). MSC labeling was achieved by 24h incubation in culture medium of ferumoxide solution (Feridex, Berlex Laboratories) and poly-L-lysine. Following labeling, the cells were washed, trypsinized, and counted by hematocytometry. Cellular loading, based on previous measurements, was estimated to be ~10 pg Fe/cell.
In vitro phantom study: An agarose gel phantom was designed using a 24-well culture plate. Wells were filled with agarose except for a 100 µL space. Cell dilutions of 2.0, 1.5, 0.75, 0.5, 0.2, and 0.1 million labeled cells were made, combined with low temperature agarose, and injected into the remaining 100 µL space in the individual wells.
In vivo rabbit study: Hind limb ischemia was induced in a New Zealand white rabbit by endovascular placement of platinum coils. Five injections (~3.5 million cells/injection site) were placed in the ischemic limb at 3 days after ischemia and imaged 24h post-injection.
MR Imaging: IRON was implemented on a 1.5T Philips Intera system with a 3D fast spin echo (FSE) acquisition. The phantom and rabbit hind limb were imaged using commercial 3D gradient echo (GRE) and FSE sequences and using the experimental IRON sequence. Imaging parameters for *in vivo & in vitro* studies were (GRE/FSE/IRON): TR=2s, TE=1.9/4.6/7.0 ms, Resolution=0.7x0.7x0.7 mm^3, Matrix=256, FSE echo spacing=4.6/7.0 ms, 22-24 echoes/TR, NSA=2, and for IRON only SPIR angle=95°, SPIR BW=100-170 Hz and TI=290 ms.
Image Analysis. On IRON images, 3D regions of interest (ROIs) of hyperintense signal were chosen by thresholding at three standard deviations above the mean of the remote agarose gel signal. Using these ROIs, volumes of signal enhancement and contrast-to-noise ratio (CNR) were measured. ROIs of hypointense signal were measured in GRE and FSE images and CNR was measured for comparison to IRON images.

Results and Discussion: In Fig. 1, signal voids were seen in GRE and FSE (A&B) images while signal enhancement was observed for IRON images (C) of the agarose phantom containing differing cell concentrations. Average CNR was 29±3 for GRE images, 29±20 for FSE images, and 53±20 for IRON images. The volume of signal enhancement was directly related to the concentration of cells (Fig. 2, R^2=0.99). In vivo rabbit images (Fig. 3) show negative contrast in GRE and FSE images and positive contrast in IRON images for three injection sites (arrows). Both in vitro and in vivo IRON images exhibit a dipole enhancement pattern consistent with theory.

Conclusions: An MRI methodology for signal-enhanced imaging of SPIO-labeled cells has been developed that enables *in vitro* and *in vivo* quantitative imaging. Because the labeled cells appear as positive contrast, the IRON method will be useful for noninvasive tracking and quantification of cellular therapeutics by enhancing the ability to distinguish the cells from other sources of image artifacts.

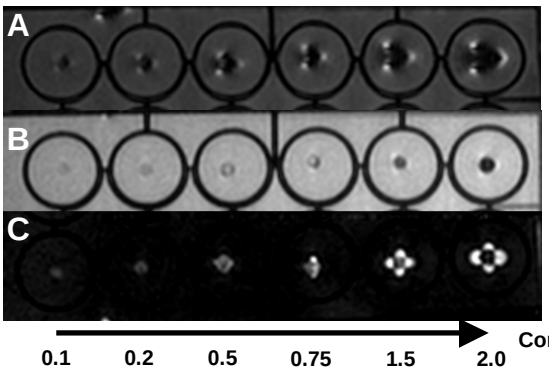

Fig 1. Transaxial (A) GRE, (B) FSE and (C) IRON images of well plates containing 0.1, 0.2, 0.5, 0.75, 1.5, and 2.0 million Feridex-labeled cells (left to right). Signal voids are observed in A and B with significant distortions in A. IRON images show good positive contrast enhancement that increases with increased concentration.

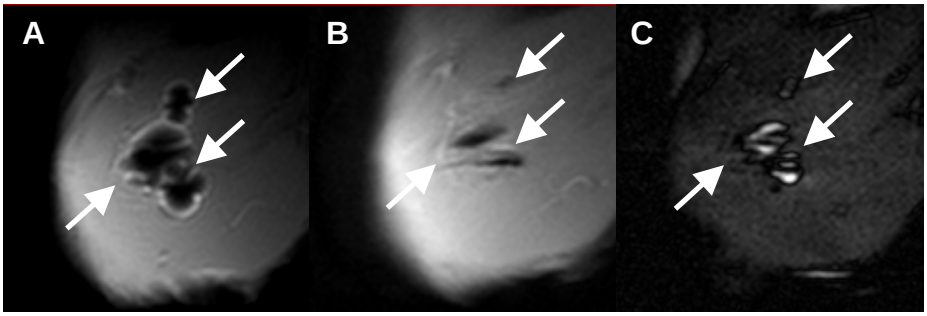

Fig 3. GRE (A), FSE (B), and IRON images of an ischemic rabbit hind limb following injection of labeled MSCs. White arrows indicate sites of injection.

References:
1. Coristine AJ et al. Proc ISMRM 2004, p.163.
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3. Pittenger MF et al. Science 1999; 284:143-7.

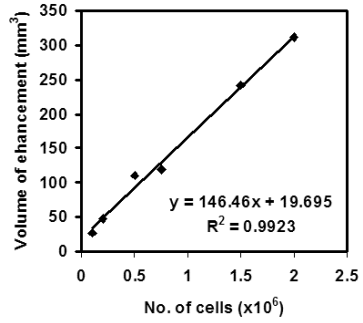

Fig 2. A high correlation is observed between the volume of enhancement measured from IRON images and the number of labeled cells in each well of the agarose phantom.