Molecular MR imaging of labeled stem cells in a mouse burn model in vivo

V. Righi[1,2], A. M. Rad[3], D. Mintzopoulos[1], A. J. Fischman[4], and A. A. Tzika[1,2]

[1]NMR Surgical Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burns Institute, Harvard Medical School, Boston, MA, United States, [2]Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Athinoula A. Martinos Center for Biomedical Imaging, Boston, MA, United States, [3]Division of Burn, Shriners Burn Institute, Harvard Medical School, Boston, MA, United States, [4]Division of Burn, Shriners Burns Institute, Harvard Medical School, Boston, MA, United States

Introduction– Recently, the interest in noninvasive novel methods for molecular imaging using MRI of clinically relevant mouse models using super-paramagnetic iron-oxide (SPIO) nanoparticle as contrast agents has increased. SPIO nanoparticles are commonly used to label cells for cellular imaging (1, 2). Several methods to generate positive contrast of magnetically labeled cells have been suggested such as: spectrally selective excitation of an off-resonance region near the labeled cells (3) and suppression of signals from non-labeled regions by dephasing (4,5). The scope of this study was to track labeled stem cells in a burn mouse model using noninvasive MRI methods for positive-contrast imaging *in vivo*.

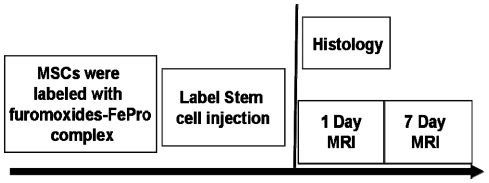

Figure 1. Experimenntal protocol illustrating the contrasct agent ingestion and the magnetic resonance time experiment.

Materials and Methods– Mesenchymal stem cells (MSCs) were obtained from bone marrow of mice and several markers (e.g. CD11b, CD106, CD90, SCA-1) were used to confirm the cells as progenitor cells (mesenchymal stem cells MSCs). MSCs were magnetically labeled with ferumoxides-protamine sulfate (FePro) complexes before injection. Mean intracellular iron was determined by spectrophotometric methods using hydrochloric acid and potassium ferrocyanide (6). MSCs (3 million cells) were intravenously injected 48 to 72 hours after thermal injury and mice were imaged 48 hours after IV injection of MSCs in a 4.7 T horizontal bore magnet (20 cm bore diameter, Magnex Scientific, using a Bruker Avance console). We acquired both positive and negative contrast MR images. Positive contrast was performed using an off-resonant imaging (ORI) method (7) implemented in a RARE sequence (also known as Fast Spin Echo, FSE) with RARE acceleration factor two. The ORI-T2ρ sequence was implemented in a RARE sequence with acceleration factor two via insertion of an MLEV-4 block with HS4 adiabatic pulses for relaxation in the rotating frame (8). The RF amplitude and the mixing time of the adiabatic pulses in the ORI-T2ρ have been optimized for *in vivo* mouse imaging (9). Selective water and fat suppression was achieved using ten-lobed sinc pulses (400 Hz pulse bandwidth for water suppression, 800 Hz bandwidth for fat suppression) followed by spoiling gradients to decohere the transverse magnetization. The water and fat suppression pulses were followed by the spin-echo imaging sequence. Negative contrast was achieved with a series of FLASH images with increasing echo time for T2* weighting, with typical values $\alpha=15^{o}$, TR = 500 ms, TE = 4, 6, 8, 12, 14 ms. Typically, 10 axial slices were acquired in the burned region (1mm thickness, 1.5mm gap, 3×3 cm FOV, 128x128 matrix size, 8 averages). Total measurement time was approx. 2.5 hr per mouse. Mice were euthanized 2 weeks after imaging and histology was performed. Histochemical determination of iron labeled cells was also confirmed by Prussian blue staining. Our results show that MSCs were efficiently labeled with ferumoxides-protamine sulfate complexes. We repeated the experiment one week after the first analysis for the same mouse.

Results– Figure 2 reports the *in vivo* images of labeled stem cells in a mouse burn model. Positive-contrast images are shown in pseudocolor, superimposed on anatomical proton-density weighted images, for representative slices from imaged mice. Both ORI-$T_{2\rho}$ and ORI images were transformed to SNR images and thresholded at the same SNR level in order to be able to provide a basis of comparison between the two imaging protocols. Care was taken to choose similar slices at the same anatomical location in all mice. Images shown are from 24h after injection (figure 1, panel 1) and after one week (figure 1 panel 2). The positive-contrast images are shown in pseudocolor, thresholded to signal greater than three in (dimensionless) SNR units, and superimposed on a T_1-weighted FLASH image. From left to right, images are: (A) ORI, (B) ORI-$T_{2\rho}$, (C) FLASH (TE = 4 ms), (D) FLASH (TE = 14 ms). For image processing, regions of interest (ROI) were drawn around the area of the burn and the total (thresholded) signal intensity was integrated within each ROI. Figure 3 show the histology for Iron labeled stem cells, the efficiency of the labeling methods was more than 95%, and was determined by manual counting of Prussian blue stained and unstained cells using a microscope. These cells can be tracked non-invasively with high sensitivity and specificity by *in vivo* MRI.

Discussion– We have performed the experiment with two methods, an ORI and an ORI-$T_{2\rho}$ method. The latter proved to have slightly higher sensitivity, which may prove to be of relevance in future work. MR images clearly showed migration and accumulation of labeled MSCs to the burn area (figure 2 panels 1A, 1B), which was confirmed by histology staining for iron labeled cells using Prussian Blue (figure 3). One week following injection of labeled stem cells, we performed MRI to see whether stem cell were still in the burn area (figure 2 panel 2A, 2B). In this case, positive contrast did not reveal any stem cells and this was confirmed by T2* weighted images with different echo times (images 2C and 2D). Our study shows the possible application of MSCs in treating burn injuries and monitoring the healing progress. We have demonstrated the feasibility of using positive-contrast to image labeled stem cells *in vivo*, in a clinically relevant burn mouse model. The results should have direct implications for assessment and longitudinal monitoring of labeled stem cells during wound healing.

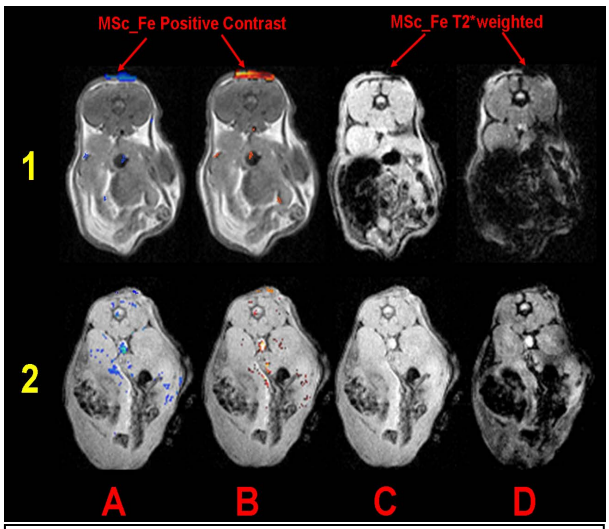

Figure 2. *In vivo* imaging of labeled stem cells in a mouse burn model, 1) after 24h and 2) one week following injection. **(A)** ORI, **(B)** ORI-$T_{2\rho}$, **(C)** FLASH (TE = 4 ms), **(D)** FLASH (TE = 14 ms). For image processing, regions of interest (ROI) were drawn around the area of the burn and the total (thresholded) signal intensity was integrated within each ROI.

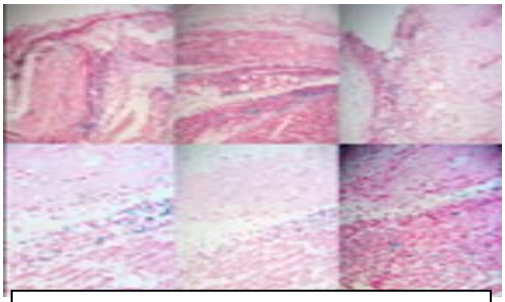

Figure 3. Histology, Prussian Blue staining for Iron labeled cells. Blue cells are MSCs injected cells.

References:

1. Rad AM . *et al.* BMC Biotechnol, 27 :9, 2009.
2. Walczak, P. *et al.* Magn Reson Med, 54:769, 2005.
3. Cunningham, CH. *et al*. Magn Reson Med 53:999, 2005.
4. Seppenwoolde, JH. *et al*. Magn Reson Med, 50:784, 2003.
5. Bakker, CJ. *et al*. Magn Reson Med, 55:92, 2006.
6. Rad AM . *et al.* Biotechniques, 43(5):627, 2007.
7. Farrar, CT, *et al.* NMR Biomed 21:453, 2008.
8. Hennig, J. *et al*. Magn Reson Med, 3:823, 1986.
9. Andronesi, OD, *et al*. Proc Intl Soc Magn Reson Med, 16:1149, 2008.