Tracking iron labeled stem cells in bone injury model using MRI

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Target Audience: Researchers and scientists interested in cell tracking using MRI, and using stem cells for treatment, especially in bone injury.
Purpose: Stem cell therapies are promising for treatment of skeletal injury[1]. It is important to study the *in vivo* behavior of implanted cells in order to implement therapies. This study aimed to develop a robust protocol to label mammalian stem cells with iron and use implanted labeled cells in combination with 9.4T MRI to study cell trafficking in a mouse bone injury model.
Methods: Embryonic stem cells (ESCs) derived from the Sv 129 mouse were used. We tested two techniques for labeling and differentiation of cell (they differ in the order). First, undifferentiated ESCs were labeled by incubating FeREX (USPIO, at a concentration of 25 µg/ml) and the transfection agent Lipofectamine for 24 hours. Then, labeled cells were differentiated towards an osteogenic lineage using a collagen substrate as reported previously for 16 days[2]. In the second technique, ESCs were first differentiated into osteogenic lineage for 16 days then labeled at the end of the differentiation interval. The mouse bone injury model is an accepted fracture model[3]. Holes were drilled (under anesthesia) in the tibia using a 0.6 mm dental drill. Labeled differentiated cells (approximately 10^5 cell) were injected in the distal hole while the other hole was left empty as control [4]. MRI RARE images were acquired using a Bruker Avance console at 9.4T and a custom built solenoid coil into which the leg was positioned. Imaging was done at day 1, 7 and 14 post-implantation. MRI parameters were; TR/TE=2000/20ms, Rare Factor=4, NA=12, Matrix=256X256, Resolution=100 x100 x500 µm. After MRI, tissues were fixed and Prussian blue histochemistry was done to confirm the location of iron-labeled cells.
Results: Differentiated ESC's did not label with FeREX (Fig. 1A), while undifferentiated ESC's showed consistent labeling based on positive staining (blue) for iron (Fig. 1B). The MRI signal intensity (SI) at the bone injury site implanted with FeREX-labeled cells increased from day 1 to day 14 post-implant, presumably due to iron loss. The SI at the control hole (drilled hole with no treatment) and in the bone marrow (BM) decreased over time (Fig. 2) as iron migrated to these sites. Iron particles in these regions were confirmed with Prussian blue staining (Fig. 3).
Discussion: The fact that differentiated cells did not uptake the label could be due to the formation of a strong extracellular matrix formed upon differentiation which might prevent the labeling material from penetrating to the cells. Labeling before differentiation was successful. MRI SI measurements showed that the iron moved from the transplantation site and traveled through the marrow. Histology confirmed the MRI findings and showed that iron was distributed among the BM and the control hole by day 14.
Conclusion: Successful ESC labeling is possible if done before differentiating the cells. FeREX labeling didn't inhibit cell differentiation. This provides a satisfactory protocol for labeling ESC's. We confirm that tracking ESC is possible in this bone injury model using MRI. This may be the first study to label, differentiate and track ESCs in a bone injury model using MRI. In conclusion, we developed a non-invasive *in vivo* tracking system that could be used to test cell-based therapies.

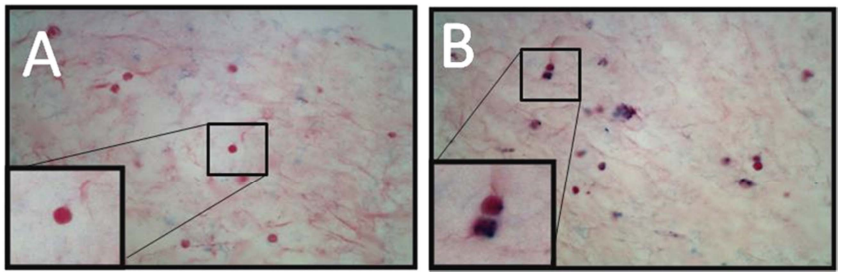

Figure 1. *Ex vivo* labeled ESCs stained with Prussian blue and nuclear fast red. A- ESCs which were differentiated before iron labeling did not stain. B- ECSs which were labeled then differentiated stained positively for iron.

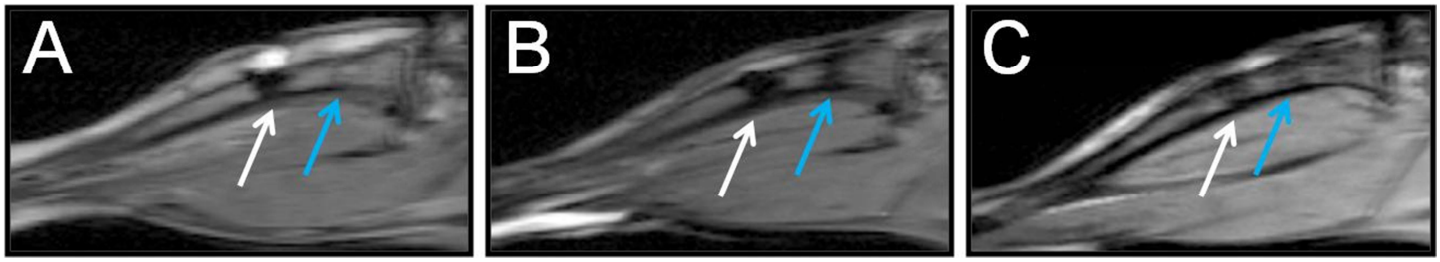

Figure 2. Example RARE MRI's acquired at day 1 (A), 7 (B) and 14 (C) post surgery with cell transplant in the mouse tibia. It shows the control hole (blue arrow) appears initially light, treatment hole with FeREX labeled cells (white arrow) appears initially dark but gets lighter as cells distribute to the bone marrow and control hole.

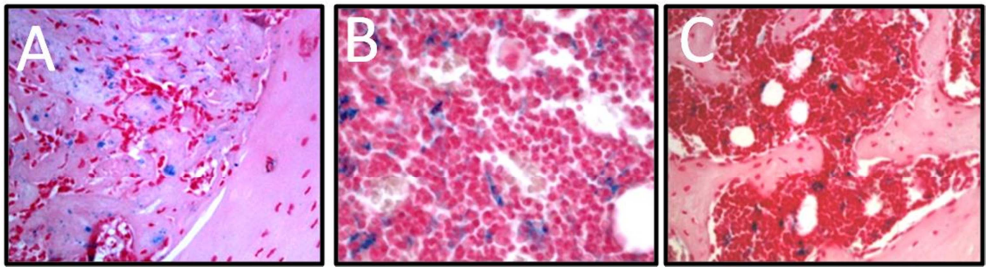

Figure 3. Histology (Prussian blue stain) done at day 14 showing the distribution of iron in the different regions of bone. (A) The treatment hole had iron in the newly formed bone. (B) The BM had iron all along the tibia. The control hole had iron only in the bone marrow spaces between the newly formed trabeculae.

References:

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