Tracking Iron Labeled Cells In Direct Transplant Models, Proceed With Caution

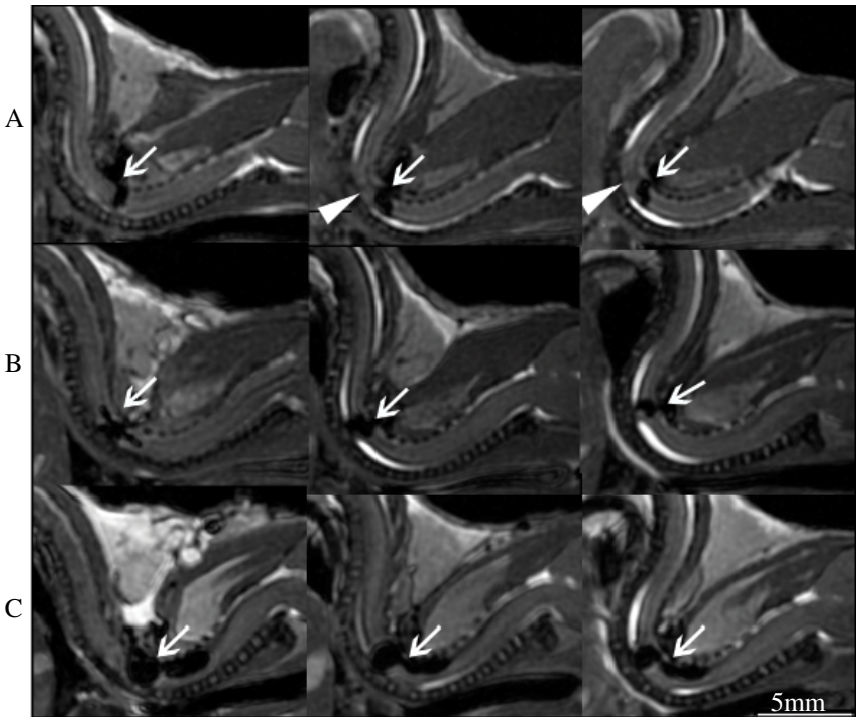
L. E. Gonzalez-Lara[1,2], X. Xu[3], K. Hofstetrova[1], A. Pniak[3], A. Brown[3,4], and P. J. Foster[1,2]

[1]Imaging Research Laboratories, Robarts Research Institute, London, ON, Canada, [2]Department of Medical Biophysics, The University of Western Ontario, London, ON, Canada, [3]BioTherapeutics Research Group, Robarts Research Institute, London, ON, Canada, [4]Department of Anatomy and Cell Biology, The University of Western Ontario, London, ON, Canada

Introduction: Stem cell labeling with iron particles allows cells to be detected by MRI and is commonly used to track stem cell engraftment. However, a concern, and potential limitation, of tracking iron labeled cells with MRI is that dead cells may be taken up by bystander cells (i.e. macrophages) and incorrectly identified as viable. Terrovitis *et al.*[1] have reported that at 3 weeks after stem cell transplants into the rat heart the MRI signal due to the iron labeled cells persisted while the histology showed the presence of iron labeled macrophages at the transplant site, and few or no viable stem cells. On the other hand, Pawelczyk *et al.*,[2] studied the transfer of the iron label from dead stem cells to bystander cells *in vitro* and reported that only 10-20% of the bystander cells became labeled and that the iron content of the bystander cells was <10% of the total iron in the labeled cells.

Without a doubt, the direct transplantation of stem cells into target tissues can lead to some iron uptake by other cells, with the ability to confuse the analysis of MRI results. With just these two, somewhat contradictory, reports it is difficult to know if this transfer of cell labels is a deal breaker for cell tracking by MRI using magnetic particles, or only something we need to be sensitive to when interpreting our data. Here we report on a study where iron-labeled stem cells were monitored *in vivo* for 6 weeks after transplant into the injured mouse spinal cord.

Methods: A clip compression spinal cord injury (SCI) was induced in C57/Bl6 mice at the level of the 4th thoracic vertebrae. Bone marrow-derived mouse mesenchymal stem cells (MSCs) were isolated by adherence and labeled with magnetic beads (SiMAG, 1μm; *Chemicell*). SCI mice received an intra-spinal transplant of (a) 30,000 live iron-labeled MSCs at day 2 (n=13) or day 7 (n=5) post SCI, (b) 30,000 dead iron-labeled MSCs at day 2 post SCI (n=4) or (c) an equivalent amount of free iron beads at day 2 post SCI (n=4). Mice were scanned at 3T using a 3D balanced steady state free precession imaging pulse sequence with TR/TE = 3.8/1.8ms, resolution of 200x200x200μm, 16 NEX and RF phase cycling. Acquisition time was 31 min. Mice were imaged on the day 0, day 2 and 1, 2, 3/4 and 6 weeks post transplantation.



Results: Live, iron-labeled MSCs appeared as a well-defined region of signal loss in the mouse spinal cord at the site of transplant (arrows). For the representative mouse shown in A, the volume of the region of signal loss decreased over time from 0.864 mm^3 at day 2 to 0.196 mm^3 at week 6. The MR appearance of dead, iron-labeled MSCs was similar to that of live MSCs (B). The region of signal loss due to the dead cells decreased over time at a faster rate compared with the live MSCs, however, at 6 weeks post transplant a substantial amount of signal loss persisted. Free iron beads in the cord also produced a prominent region of signal loss that remained at the site of transplant for 6 weeks (C). To the best of our knowledge this is the first study that demonstrates the ability to track stem cells *in vivo* in the injured mouse spinal cord.

Discussion: The signal loss attributed to transplanted stem cells can be detected and monitored *in vivo*. However, our investigation shows that whether MRI can be used to monitor and measure the survival of transplanted stem cells remains dubious; since both dead, iron-labeled cells and free iron beads produce a similar MR signal after transplantation which persists for, at minimum, 6 weeks.

Nonetheless, in other transplantation or cell transfer models that we have studied this is not the case. For instance, when iron-labeled islets are transplanted into the liver, and rejected, regions of signal loss disappear within days, as they are cleared by resident Kupffer cells. Similarly, when iron-labeled dendtitic cells migrate to the lymph node after adoptive transfer, regions of signal loss in the node disappear within days, consistent with their lifetime in the node. The direct transplantation models, as described here for the injured spinal cord, and as reported in the Terrovitis paper, may represent the worst-case scenarios for cell tracking with MRI. The method of transplantation, host tissue, cell type and extent of inflammation no doubt play important roles in the cell survival and clearance.

1. Terrovitis J *et al. Circulation* 2008**, 117**, 1555-62. 2. Pawelczyk E *et al. Stem Cells* 2008**, 26**, 1366-75.