In vivo tracking of mesenchymal stem cells in the injured mouse spinal cord.

L. E. Gonzalez-Lara[1], X. Xu[1], K. Hofstetrova[1], S. S. Ramadan[1], N. Geremia[1], A. Pniak[1], Y. Chen[1], L. C. Weaver[1], B. K. Rutt[1], A. Brown[1], and P. J. Foster[1]

[1]Robarts Research Institute, London, ON, Canada

Introduction: Recent reports show that mesenchymal stem cells (MSCs) can be induced to differentiate into neural cells.[1,2] This makes them a promising option for nerve regeneration following a spinal cord injury (SCI). MRI provides the opportunity to track the fate of MSCs transplanted into an injured spinal cord. However, in vivo imaging of experimental disease models in the mouse spinal cord is extremely difficult because of the very small size of the cord itself (1.5mm^2 in x-sec area), the extreme curvature and the challenges associated with imaging diseased mice in vivo over time. In fact, there are no published reports of the use of in vivo MRI to monitor transplanted cells in models of mouse SCI. There are only a few in vivo studies of mouse cord injuries: using ultra-high magnetic fields (9.4 T)[3], diffusion tensor imaging (4.7 T)[4], or manganese enhancement.[5]

We have implemented and optimized microimaging technology for in vivo mouse cord imaging and have applied this technology to study experimental SCI and MSCs transplantation. Our methods rely on the use of a custom-built high-performance gradient insert installed on a 3 Tesla clinical whole-body MR system and the use of a 3D steady state free precession imaging sequence. Here we show, for the first time, the ability to detect and monitor clip compression injuries in the mouse spinal cord and to track the fate of transplanted MSCs in injured mice over 4 weeks.

Methods: Whole bone marrow was acquired from EGFP+ mice (Tg(ACTbEGFO)10sb) and plated onto plastic tissue culture dishes. MSCs were isolated by adherence and labeled with magnetic beads (SiMAG, 0.75μm; *chemicell, Berlin, Germany*). A clip compression SCI was induced in genetically matched mice (K15-EGFP) at the level of the 4th thoracic vertebrae. An intrathecal transplant of MSCs was performed 48 hrs after the SCI (n=10); 7 mice received iron-labeled MSCs, 3 received unlabeled MSCs. MR imaging was performed using a 3T GE MR scanner using a custom-built gradient coil and solenoid RF coil. Mice were placed in a custom-built plastic sled to permit careful and reproducible positioning to allow sagittal imaging of the cord. The lower part of the mice body and the tail were wrapped using gauze to maintain body temperature. Mice were scanned using the 3D fast imaging pulse sequence employing steady state acquisition (3D FIESTA) (TR/TE = 3.8/1.8 ms, flip angle = 25º) at a resolution of 200x200x200μm over a 4x2.4cm FOV with 16 NEX and employing RF phase cycling. Acquisition time was 31 min. Mice were imaged at 2 days, 1, 2, 3 and 4 weeks post transplantation. Separate groups of C57/Bl6 and K15-EGFP control mice were used for image optimization steps and included uninjured (n=9) and injured but receiving no transplant (n=7).

Results: Excellent 3DFIESTA mouse cord images were obtained at 3T (Figure 1). Cord SNR was ~50. Phase cycling was effective at eliminating banding artifact. The epicenter of the SCI was visible as a region of heterogeneous signal (arrows, Figures 1 and 2). The appearance of the injury changed over time. Figure 2 shows images, acquired at day 2 and 2 weeks, of mice that received unlabeled MSCs. At 2 weeks post transplant small areas of hypointensity can be seen, due to the pathology, which were not visible at 2 days post-transplant. Figure 3 shows images, acquired at day 2 and 1 week, of mice that received iron-labeled MSCs, which appear as large, obvious regions of signal hypointensity in the cord. The region of signal loss caused by the presence of labeled MSCs diminished with time between day 2 and 1 week.

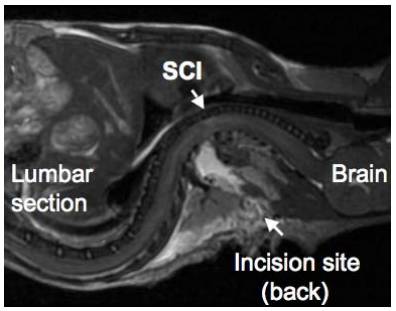

Figure 1 - Mouse spinal cord injury at T4.

Conclusion: To the best of our knowledge, this is the first report of in vivo stem cell tracking in a mouse model of SCI. Many challenges had to be overcome to generate high quality images of the mouse cord in vivo. These included the small size, extreme curvature, respiratory motion, banding artifact, and life support. Key to our success is the implementation of a fast 3D imaging sequence which is extremely sensitive to iron and has very high SNR efficiency together with the use of custom high-performance gradient coil insert technology at 3T.

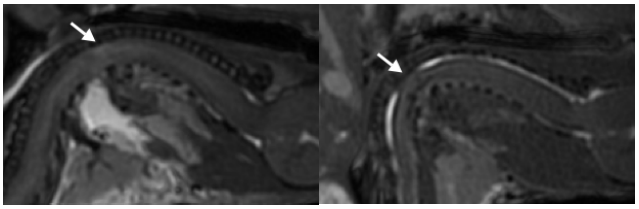
Figure 2 - Recipient of unlabeled MSCs at 2 days (left) and 2 weeks (right) after transplant.

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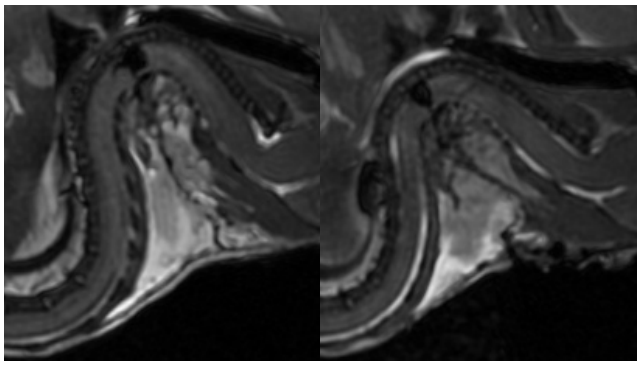
Figure 3 - Iron-labeled MSCs at 2 days (left) and 1 week (right) after transplant.