Limitations of iron-based stem cell tracking in MRI-monitoring of stem cell therapies *in vivo*

E. Tarulli[1], V. Gretka[1], A. Hoyles[2], C. Morshead[2], and G. J. Stanisz[1]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada, [2]Department of Surgery, University of Toronto, Toronto, ON, Canada

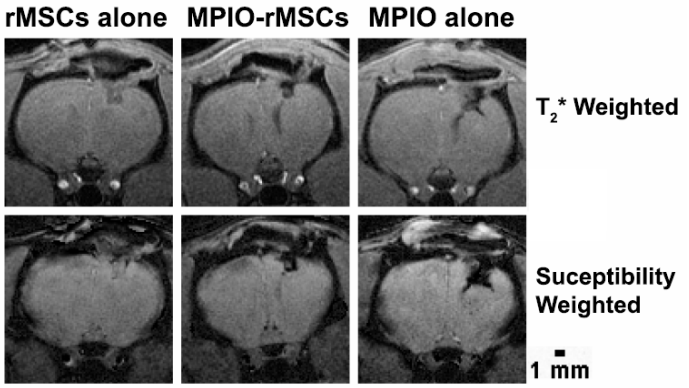
Introduction. Treatment of neurodegenerative diseases such as stroke continues to be an elusive goal for the medical community. While stem cells hold great promise for neuroregeneration, their migration and mechanism of repair are yet to be clearly defined. Literature suggests that mesenchymal stem cells (MSC) can be influenced to differentiate into neural cells *in-vitro* [1]. Several strategies have been developed for understanding their regenerative mechanism; this investigation makes use of micron-sized superparamagnetic iron-oxide (MPIO) particles for tracking MSCs in MRI. We outline some of the issues surrounding this method of stem cell tracking.

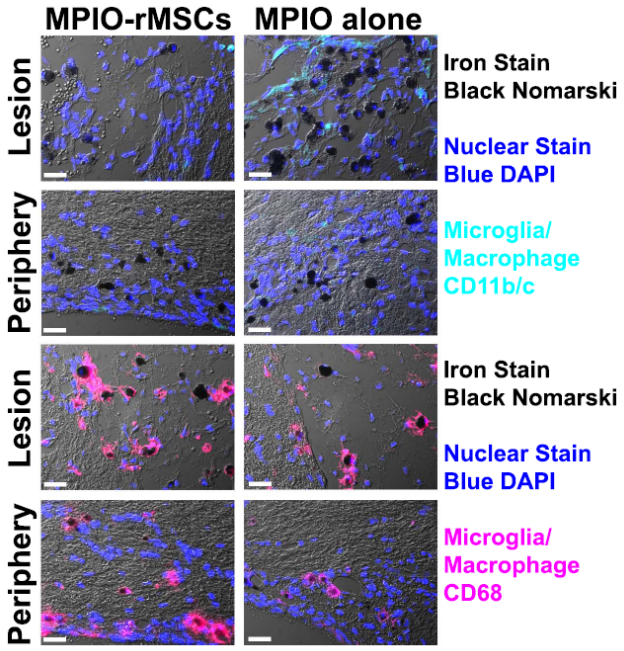
Methods. *Stroke induction*: Focal ischemia was induced in Long Evans rats (males, 200g, Charles River Laboratories) using a devascularization model [2]. An opening was made in the skull over the motor and sensory cortices. A sterile saline-soaked cotton swab was used to wipe the pia and attached blood vessels from the cortical surface, creating focal ischemia.
Derivation: Rat-derived mesenchymal stem cells (rMSCs) were harvested from bone marrow aspirates obtained from the tibias and femurs of a second group of rats 60 days post gestation.
Labeling and Injection: Cells were subsequently grown and labeled with MPIO particles to achieve ≈54 pg iron/cell. The MPIOs are composed of a divinyl benzene polymer with a mean diameter of 0.9µm immersed with 62% magnetite (Fe_3O_4) by weight and a Dragon Green fluorophore with 480 nm peak absorption and 520 nm peak emission spectra (Bangs Labs, MC05F). Animals were treated post stroke with either: ≈3×10^6 MPIO loaded rMSCs (≈54 pg iron/cell), ≈3×10^6 rMSCs without MPIO, or MPIO suspended in sterile PBS (≈160µg iron). Injections were performed via an intravenous 24 gauge catheter inserted in the tail vein 3, 7, 10, and 14 days post stroke (two animals per time point).
MRI: Rats were anesthetized (isoflurane), placed in an MR-compatible head restraint and scanned at 3T (GE Signa) using a custom-designed surface coil. Imaging was performed at various time points up to 28 days post-lesion. Each scanning session consisted of:

i) A proton weighted and T_2 weighted FSE (TE = 35,75ms, TR = 4500ms, slice thickness 1mm, matrix 256×256, 40×40mm FOV, 4 NEX)
ii) A T_2^* weighted SPGR (20º flip angle, TE = 7ms, TR = 25ms, slice thickness 1mm, matrix 256×256, 40×40mm, 4 NEX)
iii) A modified SPGR for susceptibility weighted imaging (20º flip angle, TE =25ms, TR = 43ms, slice thickness 1mm, matrix 512×256, 80×40mm FOV, 2 NEX)

Scan sessions lasted approximately 1 hour per animal.
Histology: Animals were sacrificed one day after their last imaging session. Brains were removed, sectioned, stained, and analyzed for the presence and distribution of cells, iron, microglia and macrophages.

Results. (Below) MRI of representative rat brains (14 days post stroke; injections 3 days post stroke). Signal void in the lesions periphery is observed in rMSC-MPIO treated animals. However there is also a pronounced negative contrast in the animals that received MPIOs alone. The signal voids were observed up to three weeks post injection and for all animals injected with rMSC-MPIOs or MPIOs alone at each time point (3, 7, 10, and 14 days post stroke), but were absent in unlabeled rMSCs. (Right) Immunohistochemistry and fluorescent microscopy of iron containing cells in the lesion site and periphery. Prussian Blue positive staining for the presence of iron appears black on Nomarski optics. DAPI staining in blue reveals location of cell nuclei relative to black iron deposits. Staining for microglia and macrophages (CD11b/c, CD68) in cyan and fuscia respectively. Scale bars represent 30µm. Structural and functional recovery was observed in 75% of the animals that received rMSCs.





Summary and Conclusions: We have demonstrated the ability of MPIOs as an MRI negative contrast tracking mechanism for stem cells injected into the tail vein. Moreover, we have observed structural and functional recovery in the animals that received the stem cell therapy. However the MPIO injections alone resulted in the similar patterns of signal nulling which was observed in each animal of the study. The majority of iron containing cells are CD68 positive suggesting these cells may be macrophages/microglia. Since it is expected that some of the stem cells injected will die following injection, free MPIOs can be taken up by surrounding macrophages or microglia. In this model of stroke, inflammation can persist up to four weeks post injury. The result of this study indicates that it is not possible to distinguish between iron inside injected stem cells and iron inside inflammatory cells. It remains to be seen whether different types of contrast agents exhibit similar properties.

References: (1) Zhao Lei et. al. Cell. Bio. Intl. (2007) 31(9):916–923 (2) Gonzalez C. L., Kolb B. Eur. J Neurosci. 2003;18:1950.