A Novel Strategy to Track Short-Term Stem Cell Viability and Integration Using Diffusion-Activated MRI Contrast Agents in a Murine Model of Radiation-Induced Cognitive Dysfunction

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***Target Audience*:** Stem cell research scientist and neurologist

Purpose: Traumatic brain injury (TBI) is a complex process, which usually results in neurological dysfunction.[1] Given the limited regenerative ability of the central nervous system (CNS), several stem cell-based therapies are currently being investigated.[2] In order to effectively manage therapeutic regimes, there is a need for non-invasive and clinically translatable imaging strategies capable of tracking the engraftment and survival of transplanted stem cells with high spatial and temporal resolutions.[3] In this study, we evaluated the feasibility of non-invasively monitoring the short-term viability and fate of transplanted stem cells using a dual MR contrast strategy, previously tested with nanocarriers [4, 5].

Method: Human mesenchymal stem cells (hMSCs) were dual labeled with a high molecular weight, negative (T2/T2*) contrast agent and a low molecular weight, positive (T1) contrast agent: bionized nanoferrite (BNF) particles and gadolinium-diethylene triamine penta-acetic acid (Gd-DTPA) respectively. The labeling efficiency; sub-cellular localization; cellular concentration; contrast agent maintenance and biological functioning (viability, proliferation, apoptosis and differentiation) under the optimal labeling conditions were then assessed. To evaluate the efficiency of this technique to detect graft rejection, a lentiviral based vector carrying a luciferase (luc-2) gene under the control of both EF-1α promoter and an internal ribosomal entry site (IRES) was used to stably transduce hMSCs. Following transduction, the cells were dual labeled and maintained under non-physiological conditions. Gd-DTPA release from dead cells, placed on agarose, was next monitored by T1-weighted MRI and bioluminescence imaging (BLI). Following *in vitro* studies, an image-guided radiation-induced murine model of cognitive dysfunction was developed in both immunodeficient and immunocompetent mice, to assess the efficiency of the MR activation strategy *in vivo*. Following lesion induction, the formation of the lesion was monitored by both T2-weighted and T1-weighted MRI and the neurological dysfunction assessed by contextual and cued feared memory tests. Upon lesion formation and establishment of neurological dysfunction, dual labeled luc-2 transduced hMSCs were then implanted in the mice cerebral hemispheres, contra-lateral to the lesions. Both mice groups were next monitored over a period of 30 days.

Results: *In vitro,* a strong T2 (negative) contrast was generated on T2-weighted images in both live and dead cells. However, a T1 enhancement was observed in the periphery of the dead cells on T1-weighted images (Figure 1), while no significant T1 enhancement was observed in the periphery of the live cells. This method can monitor the presence and viability of cells as few as 100,000 in a volume of 150 µl at a 0.625 x 0.469 pixel/mm spatial resolution *in vitro*. Upon brain injury induction in mice, a significant change in the permeability of the blood brain barrier of the irradiated mice was observed as early as 2-3 weeks following radiation. This change was clearly visible on contrast enhanced T1-weighted images even before it could be clearly distinguished on T2-weighted images (Figure 2a). The permeability change coincided with a change in the short-term memory (STM) of the mice as determined by the STM contextual test (Figure 2b). Five days after hMSC implantation, a strong T1 enhancement was observed around the hMSC injection site in immunocompetent mice, while no significant enhancement was observed in immunodeficient mice (Figure 3). This suggested graft rejection and was confirmed by BLI. In immunodeficient mice where the cells were accepted, a decrease in T2 was observed towards the irradiation lesion about five days after hMSC administration. This suggested the migration of the dual labeled hMSCs to the lesion from their contra-lateral injection site.

Discussion: These results collectively indicate that following graft rejection in immunocompetent mice, Gd-DTPA is released from the ruptured dual-labeled hMSCs and diffuses away from the large BNF nanoparticles into the surrounding tissues, generating a strong T1 enhancement that can serve as an imaging marker of cell death. In contrast, in viable cells Gd-DTPA remains in close proximity to BNF nanoparticles and the T2 effects of the BNF particles predominate in T2-weighted images.

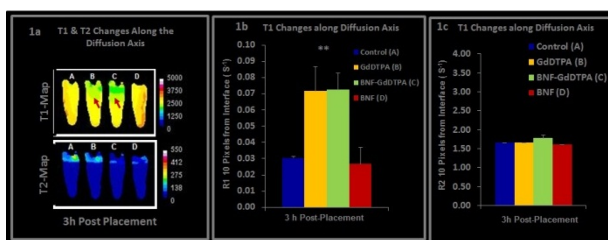

Figure 1a) *T1 & T2 changes along the diffusion axis of agarose gel pads, 3h after the placement of cells exposed to non-physiological conditions;* ***b)*** *R1 values c) R2 values, 10 pixels from the cell -agarose interface.*

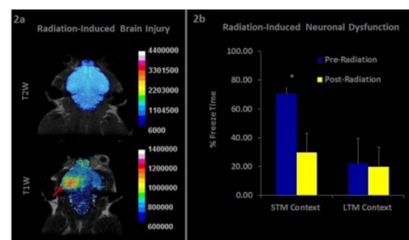

Figure 2a) *T2W & T1W images of lesion;* ***b)*** *Long and short-term memory changes before and after radiation.*

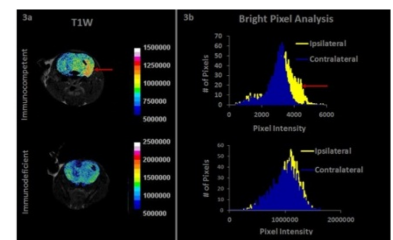

Figure 3a) *T1W images of immunocompetent and immunodeficient mice. The arrow indicates Gd-DTPA release.* ***b)*** *Bright pixel analysis of ipsilateral versus contralateral hemispheres*

Conclusion: We have developed a simple non-invasive MRI method to monitor the short-term viability and fate of the transplanted stem cells. Since this strategy employs the use of standard MRI contrast agents, it could be clinically translated to more efficiently monitor graft rejection in stem-cell based therapies. Ultimately, this could provide a means to more effectively manage stem cell-based therapeutic regimes.

References: ***1)*** McIntosh TK, et al., *Neuropath Appl Neuro* 1998, 24(4):251-267. **2)** Zietlow R, et al.,*Cell Tissue Res* 2008, 331(1):301-322. **3)** Kircher MF, et al,. *Nat Rev Clin Oncol* 2011, **8**(11):677-688. **4)** Kato Y, et al,. *Magn Reson Med* 2009, 61(5):1059-1065. **5)** Onuki Y, et al,. *Biomaterials* 2010, 31(27):7132-7138.