MR Contrast and Biological Impacts of Intracellular Superparamagnetic Iron Oxides on Human Mesenchymal Stem Cells with Hypoxic Ischemic Exposure

Jens T Rosenberg[1,2], Katelyn Sellgren[2], Michelle A Baird[3], Micheal W Davidson[3,4], Teng Ma[2], and Samuel Colles Grant[1,2]

[1]*Center for Interdisciplinary Magnetic Resonance, The National High Magnetic Field Laboratory, Tallahassee, FL, United States,* [2]*Chemical & Biomedical Engineering, The Florida State University, Tallahassee, FL, United States,* [3]*The National High Magnetic Field Laboratory, Tallahassee, FL, United States,* [4]*Department of Biological Science, The Florida State University, Tallahassee, FL, United States*

Introduction: Mesenchymal stem cells (MSCs) have gained increased interest in the field of tissue regeneration because of their availability, ease of expansion and multipotency (1-3). MSCs are known to secrete factors for regulating inflammatory processes as well as neuronal growth factors (4) in association with neural damage. A critical need in the evaluation of MSC therapies is the ability to track implanted cells longitudinally. In this study, human MSCs were evaluated after transfection with a superparamagnetic iron oxide (SPIO) nanoparticle to assess potential biological impacts of SPIO dose during long term incubation and investigate contrast permanence. In addition, cytotoxicity measurements were performed on nutrient challenged SPIO-labeled cells under normoxic and hypoxic conditions that mimic the microenvironment of ischemic stroke. To assess cell tracking capabilities at high field (11.75 T), SPIO-labeled cells were transplanted in a middle cerebral artery occlusion (MCAO) model of ischemic stroke.

Methods: Standard frozen human bone marrow-derived stem cells were acquired from the Tulane Center for Gene Therapy and were cultured following Grayson *et al* (5). Different concetrations of SPIOs (Feridex, Bayer Healthcare, Wayne, NJ) were introduced to the culture medium 24 h after plating the hMSCs (passage five) on 6-well plates, followed by 6-h exposure time. Afterward, cells were washed three times and cultured for an additional 1, 7, 14 or 21 days. Measurements of iron uptake (ICP-MS and Prussian blue staining), proliferation (MTT), colony forming ability (CFU-F), osteogenic (ALP-expression), adipogenic differentiation (adipogenic induction media), gene expression (RT-PCR) and cytotoxicity (lactate dehydrogenase, LDH release) were performed on cells labeled with direct SPIO doses. For MRI, 150,000 harvested cells were mixed with equal volume of 2% agarose and layered in a 10-mm NMR tube. Samples were imaged on an 11.75-T magnet, equipped with an Avance console (Bruker-Biospin, Billerica, MA) to quantify R_2 and R_2* relaxation using 2D spin-echo (SE) and gradient echo (GRE) sequences with increasing echo times. 3D GRE datasets (100-µm isotropic resolution) also were acquired. For *in vivo* experiments, $1x10^6$ cells labeled with carboxyfluorescein succinimidyl ester (CFSE; Sigma, MO) and SPIOs tagged with rhodamine (BioPal, MA) were injected arterially into Sprague-Dawley rats following an arteriotomy performed 1 h prior to induce a MCAO (5,6). After 48 h, the animal was sacrificed and transcardinally perfused with 4% paraformaldehyde. The head was imaged at 11.75 T using a 3D GRE sequence with a 100-µm isotropic resolution and TE/TR = 7.5/150 ms. Histology was performed on 20-µm tissue sections treated with Hoechst nuclear stain and imaged with DAPI, FITC & Texas Red filters.

Results: Data demonstrates that hMSCs readily engulf SPIOs once exposed in culture medium without transfection agents in a dose dependent fashion (Fig 1). A decrease in MRI contrast is seen for all SPIO loadings but with the highest exposure (56-µg) retaining contrast most successfully over time. There was no effect on proliferation as determined by MTT or cellular stemness as determined by CFU-F. SPIO incorporation significantly reduces ALP expression at day 7; however, it significantly up regulated calcium production by day 21 meaning that osteogenic differentiation was not inhibited even though SPIO incorporation may have down regulated ALP initially. SPIOs did not affect adipogenic differentiation ability, and no differences were seen for any primer using RT-PCR between control and initial iron loadings at days 7 and 14 days. On the other hand, SPIO labeled cells that were exposed to *in vitro* ischemic conditions (*i.e.* 2% O_2 and 2% serum) reveal an increase in cytotoxicity at 24 hrs for all iron loadings compared to normoxic conditions (Fig 2). Under *in vitro* ischemic conditions, the cells also show a statistical increase in vulnerability between unlabeled cells and the two highest initial SPIO loadings at 24 h. In Fig 3, rhodamine-tagged SPIOs (red) are located in the perinuclear region within hMSCs (CFSE green) in monolayer culture. Once hMSCs were injected into the MCAO rat, *ex vivo* MRI reveals localization of labeled cells to the ipsilateral side of the induced stroke. Fluorescence shows that CFSE and SPIO labeled cells are present within the stroked hemisphere and that ipsilateral nuclei (blue) decorated with SPIOs are 2.7x more prevalent than the contralateral.

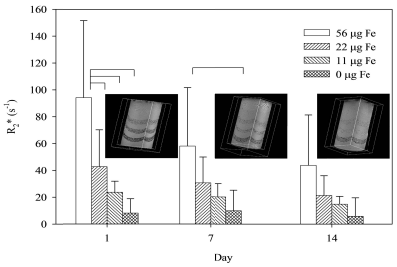

Fig 1: R_2* with GRE images inserted for respective time point and initial SPIO exposure (from bottom: 56, 22 and 11 µg Fe).

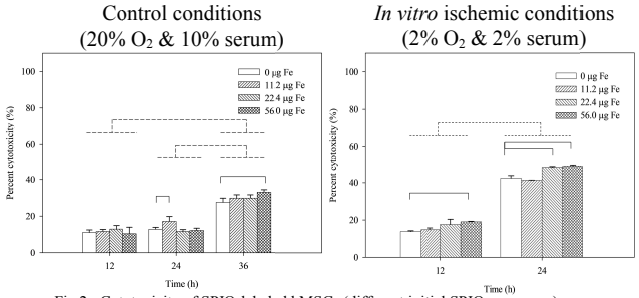

Fig 2: Cytotoxicity of SPIO-labeled hMSCs (different initial SPIO exposures) during control normoxic/nutrient conditions as well as hypoxic ischemic conditions. Statistical differences are indicated by dashed and solid brackets ($p<0.05$)

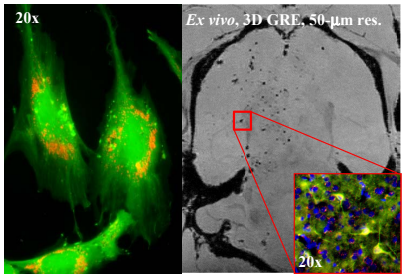

Fig 3 Left: Confocal image of CFSE-SPIO-Rh labeled hMSCs. Right: MRI with signal voids from iron labeled cells. Flourescent image show hMSCs (bright green).

Discussion: This study shows that labeled hMSCs maintain internalized SPIOs over an extended culture period as seen with a tissue phantom and with minimal impact on stemness, proliferation and differentiation. However, MRI measurements do reveal reductions in contrast over prolonged periods, likely due to reduced intracellular iron by dilution during cell division, and contrast persistence was best for the highest initial SPIO exposure. This finding has repercussions for the extended *in vivo* tracking of labeled hMSCs and permanence of long term contrast. Furthermore, cells exposed to *in vitro* ischemic conditions show increased cytotoxicity at 24 h and complete death at 36 h, with increased toxicity for the higher iron loadings compared to control. As such, the viability of challenged cell implants must be considered when applying intracellular contrast agents for tracking hMSCs *in vivo* because hypoxic-ischemic microenvironments may challenge cell performance similarly. With arterial injection into an *in vivo* MCAO model, hMSCs initially exposed to a mid-range concentration of SPIO (22-µg effective Fe) created enough susceptibility induced contrast to be detected with MRI. These signal voids extend beyond the location of the single cells due to the microscopic field gradients created by the SPIO. The presence of ipsilateral labeled hMSCs was confirmed by the fluorescent CFSE and rhodamine signals two days after injection. However, it should be noted that nuclei not associated with CFSE also displayed rhodamine decoration, indicating that SPIOs were engulfed by endogenous cells likely as a result of hMSC death and macrophage/microglial actions. Ongoing work is focused on evaluating techniques to pre-condition hMSCs as a means of enhancing the permanence of these cell implants in ischemic lesions.

Acknowledgement and References: All work has been conducted in accordance with FSU Animal Care and User committee. Data was acquired at the FAMU-FSU College of Engineering with funding support from the American Heart Association (SE division).

1. Bernardo ME et al. Cancer Res 2007 **2.** Pittenger et al Science 1999 **3.** Friedenstein AJ Cell Tissue Kinet 1970 **4.** Pavlichenko N et al Brain Res 2008 **5.** Grayson WL et al Biotechnol Prog 2004 **6.** Longa EZ *et al Stroke* 1989 **7.** Uluç K *et al* J.Vis.Exp. 2011