Dual Iron/Fluorine Cell Tracking: Monitoring the Fate of Human Stem Cells and the ensuing Cellular Inflammatory Response

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Background: Regenerative stem cell therapy is anticipated to revolutionize modern medicine. Mesenchymal stem cells (MSC) are a leading candidate for clinical trials due to their multipotent properties and presence in adult tissue. However, before treatment can progress to the clinic there are still many questions that must be answered concerning the immune rejection of transplantations. Cellular MRI offers a tool to non-invasively track the fate of stem cells and improve treatment outcomes. Recently, a study by Hitchens et al. (Magn Reson Med, 2014) showed it was possible to combine iron oxide and perfluorcarbon cell labeling to distinguish co-labeled and adjacent cells with $^{1}H/^{19}F$ MRI.[1] In this study we build on this technique by using dual iron/fluorine MRI to monitor two distinct cell populations: (i) MSC, labeled *in vitro* with ^{19}F prior to implantation and, (ii) phagocytic immune cells, labeled *in situ* through administration of intravenous (IV) iron.

Methods: Immune competent, C57Bl/6 mice were implanted intramuscularly with $1.05x10^6$ human MSC (hMSC) labeled with a red fluorescent perfluorocarbon emulsion. This model was chosen to produce an acute immune response. 7 days after hMSC implantation, mice received an IV tail vein injection of ultra-small iron oxide nanoparticles (USPIO) at a dose of 4µg Fe/g. On day 8, 4 hours prior to imaging, the mice received 10ng of lipopolysaccharide (LPS) IP to stimulate macrophages. Proton (^{1}H) and ^{19}F images were acquired at 9.4T with a dual-tuned $^{1}H/^{19}F$ mouse body coil using a 3D-balanced steady state free precession (bSSFP) sequence. Image resolution was $1x1x1mm^3$ for ^{19}F and $200x200x200\mu m^3$ for ^{1}H. Total scan time was under 90 minutes. Mice were anaesthetized with isoflurane and imaged four times, up until day 8. NMR was performed using $2x10^6$ ^{19}F-labeled hMSC in order to determine the intracellular loading. Quantification of the ^{19}F-labeled cells was performed by measuring the signal in the region of interest and in a reference of known concentration using Voxel Tracker software. Following imaging, mice were sacrificed and tissues removed for immunohistochemistry (IHC).

Results/Discussion: On the day of implantation (day 0) the ^{19}F-labeled hMSC were visible in all mice and *in vivo* ^{19}F-MRI quantification agreed strongly with the number of implanted cells (figure 1A). The site of implantation was visible in the proton image as a hyper-intense region, due to the additional fluid. On both day 3 and 6, the hyper-intense region was still visible, and localized to the ^{19}F signal from the hMSC (figure 1B). The ^{19}F signal gradually decreased between days 0 and 6, (figure 2, although the change was not statistically significant). Proton images obtained on day 8, 24 hrs after the IV injection of USPIO, showed a large region of signal void at the transplantation site (figure 1C). Coincident with this was a significant drop in the ^{19}F signal at the transplant site. The ^{19}F signal on day 8 was decreased by 63% from day 6. The observation of signal loss in proton images after IV USPIO is consistent with the infiltration and accumulation of iron-labeled macrophages at the implant site. Likewise, signal loss was also observed in the lymph nodes. Other regions of low signal intensity observed in proton images were present before and after the IV USPIO injection (ie. bone, air). Many studies have proven the utility of IV iron for macrophage tracking.[2] In addition, the transplant model we used would be expected to cause an acute cellular inflammatory response; host macrophages migrate into transplants in response to cell death and inflammation. These data demonstrate the ability to monitor two different populations of cells in a single scanning session with the same pulse sequence. It is noteworthy that the signal loss, generated by what we presume to be iron-positive macrophages, did not quench the entire ^{19}F signal. IHC is underway to confirm and quantify the presence of hMSC and macrophages in tissue.

Significance: This study is the first reported application of dual iron and fluorine MRI agents for tracking different cell types *in vivo*. We have shown that it is possible to non-invasively monitor implanted stem cells with ^{19}F-MRI, while simultaneously monitoring the influx of iron-labeled immune cells. This imaging approach could allow for the detection of failed stem cell transplants by revealing the influx of iron-labeled macrophages. We anticipate the capability to track multiple cell types with a single modality will be of interest to researchers investigating cellular therapeutics.

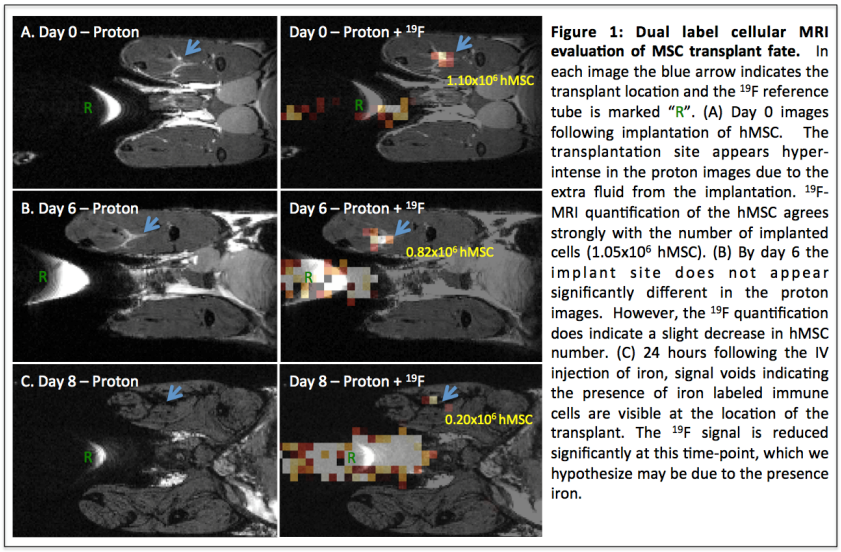

Figure 1: Dual label cellular MRI evaluation of MSC transplant fate. In each image the blue arrow indicates the transplant location and the ^{19}F reference tube is marked "R". (A) Day 0 images following implantation of hMSC. The transplantation site appears hyper-intense in the proton images due to the extra fluid from the implantation. ^{19}F-MRI quantification of the hMSC agrees strongly with the number of implanted cells ($1.05x10^6$ hMSC). (B) By day 6 the implant site does not appear significantly different in the proton images. However, the ^{19}F quantification does indicate a slight decrease in hMSC number. (C) 24 hours following the IV injection of iron, signal voids indicating the presence of iron labeled immune cells are visible at the location of the transplant. The ^{19}F signal is reduced significantly at this time-point, which we hypothesize may be due to the presence iron.

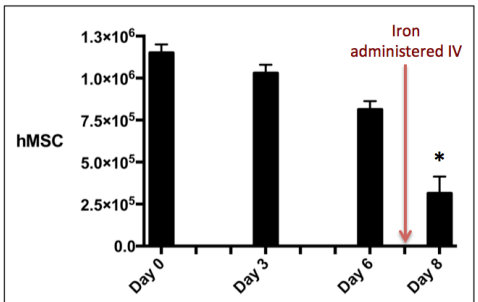

Figure 2: *in vivo* ^{19}F-MRI quantification of hMSC. Following implantation of $1.05x10^6$ hMSC, ^{19}F-MRI was used to quantify the number of cells over 8 days. Significance from the previous imaging time point is denoted by *. On day 7, iron was administered IV resulting in a large ^{19}F signal decrease on day 8.

References: 1. Hitchens TK, Liu L, Foley LM, Simplaceanu V, Ahrens ET, Ho C. Combining perfluorocarbon and superparamagentic iron-oxide cell labeling for improved and expanded applications of cellular MRI. *Magnetic Resonance in Medicine.* 2014.

2. Dousset V, Delalande C, Ballarino L, Quesson B, Seilhan D, Coussemacq M, Thiaudiére E, Brochet B, Canioni P, Caillé JM. In vivo macrophage activity imaging in the central nervous system detected by magnetic resonance. *Magnetic Resonance in Medicine.* 1999;41:329-33