Feasibility of accurately determining cell number by ^{19}F MRI and the impact of cellular rejection, inflammation and transfer of label

Jeff M Gaudet[1,2], Emeline J Ribot[3], Yuhua Chen[1], Kyle Gilbert[4], and Paula Foster[1,2]

[1]*Imaging Research Laboratories, Robarts Research Institute, London, Ontario, Canada,* [2]*Medical Biophysics, University of Western Ontario, London, Ontario, Canada,* [3]*Centre de Resonance Magnetique des Systemes Biologiques, Universite Bordeaux, France,* [4]*Centre for Functional and Metabolic Mapping, Robarts Research Institute, London, Ontario, Canada*

Background: Stem cell therapy has the potential to revolutionize modern medicine and clinical trials are already underway. Still, there are questions about which parameters (numbers of cells, transplant route, timing) produce the best transplantation protocols. Most cellular MRI studies have used iron labels to image stem cells. This approach provides excellent cell detection sensitivity, but suffers from low specificity and quantification of labeled cells is difficult. Fluorine-19 (^{19}F) MRI can address this limitation because of the potential for unambiguous detection and accurate quantification, since mammalian tissues have negligible ^{19}F. In this study we use MRI to detect mesenchymal stem cells (MSC) labeled with a ^{19}F agent to track their fate over time *in vivo*. Here we present two key findings that advance our understanding of ^{19}F -based cell tracking: (i) ^{19}F MRI can be used to quantify cell number *in vivo*; we show validation of this by microscopy and (ii) the ^{19}F cell labeling agent can be transferred to macrophages in conditions where transplanted cells undergo cell death, and this signal can remain detectable by MRI.

Methods: We used two transplantation models (i) a xenograft - human MSC (hMSC) implanted into immune-compromised nude mice (n=6) and (ii) an allograft - mouse MSC (mMSC) implanted into immune-competent C57Bl/6 mice (n=7). MSC were labeled with a red fluorescent perfluorocarbon emulsion (Cell Sense). 1.5x10^6 ^{19}F-labeled hMSC were implanted and 2x10^6 mMSC were implanted; mMSC were also GFP+. 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 1x1x2mm^3 for ^{19}F and 200x200x200µm^3 for ^{1}H. Total scan time was under 90 minutes. Mice were anaesthetized with isoflurane and imaged four times, up until day 17. 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. NMR was performed using 2x10^6 ^{19}F-labeled hMSC and mMSC in order to determine the intracellular loading. Some mice were sacrificed for histological analysis of tissue to validate our MRI data. Tissues were examined from one mouse in each group at day 0 and day 17. Fluorescence microscopy and immunohistochemistry (IHC) were performed on the same tissue slice for precise visualization of the location of labeling agent and cells.

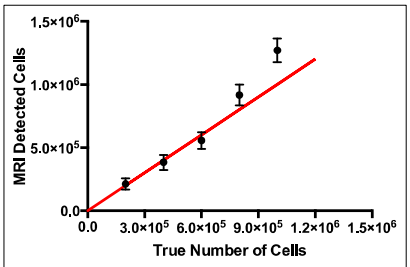

Figure 1: Verification of Quantification Accuracy using Cell Sense labeled cells.
Cell pellets were collected ranging from 2x10^5 to 1x10^6 hMSC. ^{19}F-MRI and ^{19}F-NMR was performed on the pellets alongside a reference tube of known ^{19}F concentration. Voxeltracker™ software was used for quantification. The red line represents the ideal result of a 1:1 correlation.

Results/Discussion: We tested the accuracy of ^{19}F-MRI quantification using a phantom made of 5 different cell pellets (Fig1). Precision was found to be very good, with a linear correlation value of R^2= 0.87. ^{19}F images at day 0 showed signal at the site of implantation in all mice (Fig2). Quantification of this signal produced values for cell numbers that correlated well with the number of cells implanted. The transplanted cells were clearly visible at the implant site in tissue examined at day 1, for both models (Fig3). Figure 3A shows an overlay of green/red fluorescence from the GFP+ mMSC and the red Cell Sense agent at the implant site. Inflammation was detected at day 1 in both models.

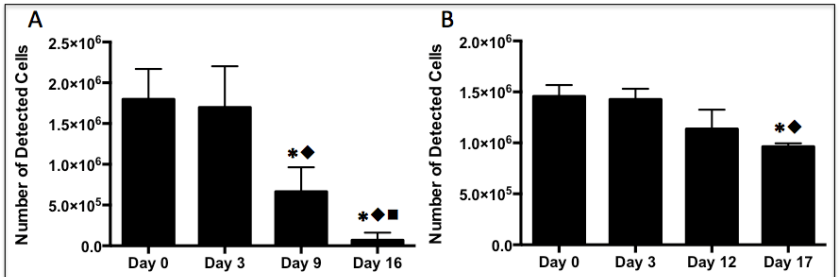

Figure 2: Comparison of ^{19}F-labelled cell detection in two transplantation models over time
(A) Following implantation of 2x10^6 mMSC, ^{19}F-MRI was used to quantify the number of cells remaining over 16 days. By day 16, only 2 mice had any detectable signal remaining. A significant difference from day 0 is denoted by *, from day 3 by ◆,and from day 9 by ■. (B) The number of detectable cells over the same time period following a transplant of 1.5x10^6 hMSC. Statistical significance is denoted in the same way as A.

Over time the ^{19}F signal decreased (Fig2). In the allograft model no ^{19}F signal was detected in 4/6 mice at day 16. This was validated by an absence of GFP signal and only very limited red fluorescence. This reflects the death and clearance of the implanted MSC. The minimum number of cells we detected in these mice was 7x10^4 at day 16. In the xenograft model ~60% of the ^{19}F signal persisted at day 17. Many Cell Sense-positive cells were detected at the implant site by microscopy (3D). However, F4/80 IHC revealed that these remaining labeled cells were macrophages. Therefore, in the xenograft mice, tracking the fate of hMSC is complicated by the transfer of ^{19}F agent, from hMSC to bystander cells. The strength of the cellular rejection in a xenograft is expected to be greater than for an allograft since more antigens are recognized as foreign, which explains why large numbers of macrophages are present. In the allograft mice a small number of F4/80 positive macrophages were found to be associated with the little red fluorescence observed at day 17 (3B). However, these cells were undetectable by ^{19}F MRI. This is an example of how the lower sensitivity to ^{19}F-labeled cells may have some benefit; had the cells been labeled with iron oxide nanoparticles the resulting signal loss would likely be detected despite the low number of cells.

Significance: This study shows, for the first time, the correspondence between cell number, determined from NMR and ^{19}F MRI, and microscopic visualization of cells by fluorescence and IHC. In the short-term after transplantation, or under conditions where cells are efficiently cleared, as in allografts, one can expect accurate quantification of the transplanted cell population. Where there is substantial cell death and associated inflammation, as with xenotransplantation, the transfer of label to macrophages will confuse the interpretation of the long-term fate of cells.

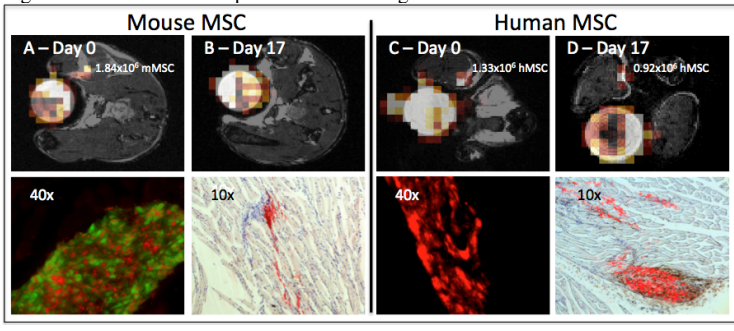

Figure 3: ^{19}F-MRI of labeled MSC corresponds to histological and fluorescent images
Representative MRI and histology images from the two mouse models. The MRI images were produced by overlaying the ^{19}F image on to the corresponding ^{1}H image to provide anatomical context for the detected signal. (A) Day 0 allograft implantation of 2x10^6 mMSC. Fluorescence microscopy was used to show the red fluorescent ^{19}F-agent sequestered within the GFP+ mMSC. (B) By day 17, there is no detectable ^{19}F signal using MRI. Fluorescence microscopy revealed no GFP+ cells. A small amount of the ^{19}F-agent can still be seen at the site of implantation. Immunohistochemistry staining with the anti-F4/80 antibody reveals the presence of macrophages in the same area as the ^{19}F agent. (C) Day 0 xenograft transplant of 1.5x10^6 hMSC. Fluorescence microscopy was again used to detect the ^{19}F-agent at the site of implantation. (D) On day 17, in the xenograft model ^{19}F is still detectable with MRI and fluorescence microscopy indicates more ^{19}F-agent remains at the implantation site compared to the allograft model. Large numbers of macrophages were seen corresponding to the location of the ^{19}F-agent.