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

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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-$^{19}$F (19F) 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). 1.5x10^7 $^{19}$F-labeled hMSC were implanted and 2x10^6 mMSC were implanted; mMSC were also GFP+. Proton (1H) and $^{19}$F images were acquired at 9.4T with a dual-tuned $^{1H}$/19F mouse body coil using a 3D-balanced steady state free precession (bSSFP) sequence. Image resolution was 1x1x2mm for $^{19}$F and 200x200x200μm3 for 1H. Total scan time was under 90 minutes. Mice were anesthetized 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 2x10^7 $^{19}$F-labeled hMSC and mMSC in order to determine the intracellular label. 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.

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 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.

![Image](image-url)