MRI of Single SPIO Labeled Cells in Mice on a 1.5T Clinical Scanner

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Introduction: MRI of superparamagnetic iron oxide (SPIO) labeled cells has become a powerful tool for tracking cell migration in vivo in animals and humans. In vivo cell tracking with MRI, however, has to date been limited to studies of large groups of cells (500-50,000 cells)[1,2]. The ability to track smaller groups of cells or single cells in vivo would represent a significant advance in cell tracking technology by providing a more accurate picture of the heterogeneity in cell behavior that exists within a population of cells. A number of groups have now demonstrated that MRI has the sensitivity to detect single cells in vitro[3] and ex vivo[4]. The high field strengths (7T) and lengthy scan times (2-16 hrs) that were used in these studies, however, have precluded their application in vivo. Recently, we have demonstrated that single cells can be efficiently detected in vitro with the 3DFIESTA pulse sequence at 1.5 T and at a resolution and scan time that can be achieved in vivo in small animals[5]. In the current work we use this technique to image single cells ex vivo in mouse brain and provide optical validation of single cell detection. This work represents the first step towards single cell detection in vivo and opens up the exciting possibility of tracking single cells at clinically relevant MRI field strengths.

Methods: ***Cell Culture:*** J774 cells, a murine macrophage cell line, were labeled with SPIO (Feridex IV; Berlex Laboratories, Wayne, NJ) and stained with the fluorescent dye DiI. The mean cellular SPIO content, assessed using a susceptometry technique described elsewhere[6], was measured to be ~28 pgFe/cell. ***Animal Preparation:*** Mice were anesthetized with an intraperitoneal injection of Ketamine (100mg/ml) Xylazine (20mg/ml) mixture with a dose of 0.03ml/10g). 5,000 SPIO labeled J774 cells were injected via an intracardiac injection into the left ventricle to deliver cells to the brain. Animals were sacrificed within 2 hours of injection and the brains removed and fixed in 3.75% formalin for 24 hours, ***MRI:*** Excised mouse brains were immersed in Fluorinert (FC-77, 3M), a perfluorinated liquid with no MR signal, and scanned on a 1.5T GE CV/i MR scanner using a custom-built gradient coil (maximum gradient strength 600mT/m, peak slew rate 3000T/m/s) and a customized solenoidal radiofrequency coil (2 cm diameter). Brains were scanned using a 3DFIESTA pulse sequence (TR/TE 7/3.4ms, flip angle 30°, (100x100)μm^2 in plane resolution, 200 µm thickness, scan time=~35 minutes). Voxels containing SPIO labeled cells appear as areas of low signal intensity (discrete "signal voids"). For each signal void, the contrast was calculated by taking the difference between the signal from the central voxel of the void (S_{cell}) and signal from background tissue containing no cells (S) and dividing by the background signal: $\Delta S/S=(S-S_{cell})/S$. ***Confocal Microscopy:*** Contiguous 25 µm frozen sections were collected in the rostral portion of the brain. Each section was imaged at low magnification for DiI labeled cells using a Zeiss 510 laser scanning confocal microscope. Inherent tissue autofluorescence was used to give reference to the location in the brain each cell was located. Finally, high magnification optical z-sectioning (0.7 µm optical section) of regions containing DiI-labeled cells was performed to confirm the presence of single cells.

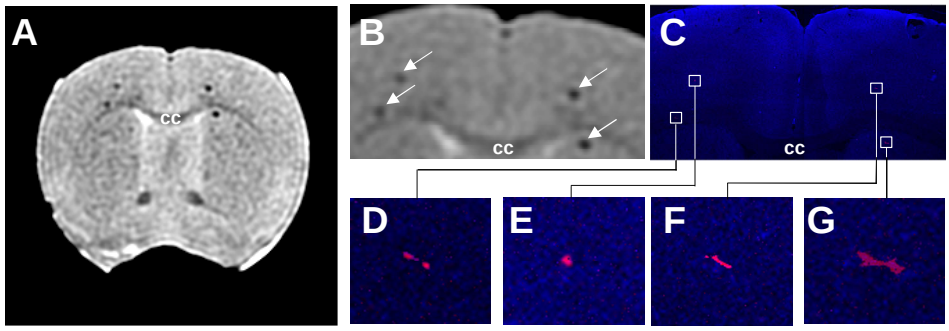

Figure 1: MR/Optical correlation of cells in mouse brain. (A) Ex vivo MRI of mouse brain showing four signal voids corresponding to SPIO labeled cells in the area around the corpus callosum (cc). (B) Magnified view of MRI showing the four signal voids (white arrows). (C) Low magnification projection through several confocal slices corresponding to the MR slice shown in (B): the blue channel is tissue autofluorescence to highlight brain anatomy, Four regions containing DiI labeled cells (red channel) were visible in this projection and have been highlighted with white boxes and shown in low resolution magnified views (D-G). These four areas correlate well with the four signal voids visualized in the MR slice.

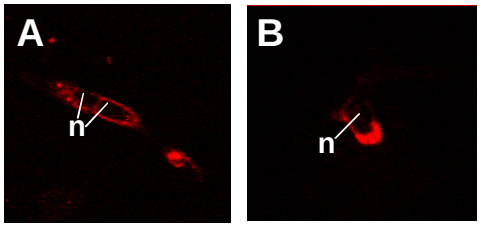

Figure 2: Validation of single cell detection. High resolution magnified optical z-section (single slice) through the cells shown in Fig. 1D and 1E (Fig. 2A and 2B respectively). The DiI fluorescence is localized to the cytoplasm of cells and the nuclei (n) appear as oval regions of no fluorescence. The cells are elongated as they are trapped in small capillaries. The number and size of nuclei indicate that these cells are single cells (the nucleus in 2A is dividing). A similar analysis of cells shown in Fig. 1F and 1G indicate that these areas contain two cells (data not shown).

Results: Ex vivo MRI of brains from mice injected with 5,000 SPIO labeled macrophages via intracardiac injection, demonstrate discrete signal voids corresponding to SPIO labeled cells throughout the brain volume (~100 voids/brain). The contrast in FIESTA images from voxels containing single cells ($\Delta S/S$) has been previously shown to be proportional to the amount of SPIO (and number of cells) within the voxel[7]. The average $\Delta S/S$ from signal voids throughout the brain was 0.35 (σ^2=0.01) which was consistent with measurements of $\Delta S/S$ for voxels containing single cells with ~29pgFe in vitro (data not shown). To validate single cell detection, co-registration of signal voids on MR images with DiI fluorescence from confocal microscopy images were performed (Figure 1) and high resolution optical z-sectioning of each region containing DiI labeled cells was used to demonstrate the presence of single cells (Figure 2).

Discussion and Conclusions: Macrophages labeled with SPIO were used in these experiments because of the relative ease and wide range of SPIO loadings that can be achieved in this cell type. The results of this work, however, are easily applicable to studies of cells other than macrophages as the SPIO loading used in this experiment is similar to what has been achieved in other cell types including cancer and stem cells with minimal impact to cell function[8]. This work demonstrates that single SPIO loaded cells can be detected ex vivo at a resolution, SNR, and scan time that can easily be achieved in vivo in small animals. This opens up the exciting possibility of performing single cell tracking and analysis in vivo, non-invasively, and longitudinally in a single animal. It is also noteworthy to mention that we met the requirements for single cell imaging on a 1.5 T clinical scanner, and while a gradient coil insert was used to achieve the high resolutions needed to detect single cells, it is interesting to speculate that single cell tracking should one day be possible in humans.

References: [1] Hoehn et al. PNAS 99(25):16267-16272, 2002, [2] Bulte et al. Nat Biotehnol. 19(12):1141-7, 2001, [3] Dodd et al. Biophys J 76(1 Pt 1): 103-109, 1999 [4] Shapiro et al. PNAS 27;101(30):10901-6, 2004, [5] Foster-Gareau et al. MRM 49(5):968-971, 2003, [6] Bowen et al. 48(1):52-61, 2002, [7] Heyn et al. MRM in press, [8] Arbab et al. Radiology 229(3):838-46, 2003.