Positive Contrast Labelling of SPIO Loaded Cells in Cell Samples and Spinal Cord Injury

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Introduction

Recent work [1] has demonstrated the use of positive contrast labeling in tracking catheter-based paramagnetic markers, such as those used during endovascular interventions. This "white-marker" technique exploits the natural dipolar field surrounding paramagnetic markers by introducing a dephasing gradient on the slice-select axis to spoil signal across the sample except in regions where the local gradients surrounding the paramagnetic marker are of the right amplitude and orientation to refocus the lost signal. The resulting image signal is void everywhere except in a small volume surrounding the marker, which is bright.

Cellular imaging, meanwhile, is an emerging field that uses high-resolution magnetic resonance microscopy (MRM) along with superparamagnetic iron oxide (SPIO) based contrast agents to visualize individual or small numbers of cells. The presence of cellularly compartmentalized iron oxide is indicated by local signal hypointensities in T2 or T2* weighted images. We have recently shown that a fully-balanced, clinical SSFP imaging sequence (3D FIESTA, GE Medical Systems) can be used to image *individual* SPIO loaded cells at 1.5T [2]. A disadvantage of this form of cellular imaging is that susceptibility-induced artifacts from macroscopic field inhomogeneities (e.g.: at tissue interfaces) can cause regions of signal void that appear similar to cellularly-compartmentalized iron oxide sources and complicate the unequivocal recognition of labeled cells. At high main magnetic field strengths, which are most commonly used for MRM, the sensitivity to susceptibility-induced artifacts from such macroscopic field inhomogeneities is increased.

In this paper we show that the white-marker (WMk) technique can be successfully adapted to produce positive contrast from SPIO-labeled cells, and presents a novel method for distinguishing signal voids created by cells from sources that are weaker field perturbers. The rat model of SCI was chosen to illustrate this characteristic and to provide proof-of-principle for our positive-contrast cellular imaging technique.

Methods

Cell Loading: Superparamagnetic iron-oxide loaded microspheres (0.9 μm diameter) were obtained from Bangs Laboratories (Fishers, IN). The particles are composed of a divinyl benzene matrix and contain 63.4% magnetite iron oxide (wt. %). B16-F10 cells, a melanoma cell line derived from C57BL/6J mice, were maintained in Minimum Essential Medium Alpha Medium and 10% FBS (Gibco). To label cells with microspheres, B16-F10 cells were plated in 6-well culture dishes ($5.0x10^5$ cells/well) and incubated with various concentrations of microspheres for 24 hours. Cells were washed of excess microspheres, trypsinized, and analyzed for Fe content using a susceptometry technique - the Reilly-McConnell-Meisenheimer (RMM) method - modified to use multi-echo imaging [3]. Some cells were then used to prepare cell phantoms. These were prepared by layering 20-60 cells in a single monolayer in 2% gelatin (w/w) between two layers of 8% gelatin (w/w) in an optically transparent 350 μl ELISA microwell. Cellular iron loadings were 30 pg/cell.

Tissue Model: Spinal cord injury was induced at T4 using a clip compression model in a rat specimen. Feridex was injected intravenously 3 days after surgery and 24 hours later, the animals were perfused and the cords were surgically removed for *ex vivo* imaging.

MR Imaging: All samples were scanned on a 1.5 T clinical scanner and a high performance gradient insert set with a maximum gradient strength of 600 mT/m and a maximum slew rate of 4000 T/m/sec. 3D FIESTA, SPGR, and WMk SPGR sequences were used, acquiring 100x100x200 um^3 voxels. Scan times averaged 30-40 minutes, with 4 averages, TE 5-8 ms, TR 15-18 ms, and a BW of 21-31 kHz. For the white marker sequence, an unbalanced slice-select diffusion gradient area of 8000-14,000 G*μs/cm was used, depending on the sample.

Results and Discussion

A slice was chosen from the SPGR and WMk SPGR 3D data sets to illustrate the refocusing nature of the sequence. Figure 1 shows a cross-sectional slice of the gel phantom with an overlain image in c). There is excellent correlation between signal loss in a) and signal refocusing in b).

A comparison of the images of the injured cord specimen shows that this method eliminates unwanted signal loss due to the laceration at the epicenter of the injury and also eliminates signal due to hemorrhage and edema, which can normally obscure cell detectability (Figure 2).

In trying to image individual or small numbers of cells *in* or *ex vivo*, one of the greatest challenges is determining whether a given source of signal loss is the result of inherent tissue characteristics or the contribution of cellularly-compartmentalized iron. Currently, the only accepted confirmation is through histological, or other destructive techniques, which prevent longitudinal studies. The WMk technique is a potential non-invasive alternative to these methods.

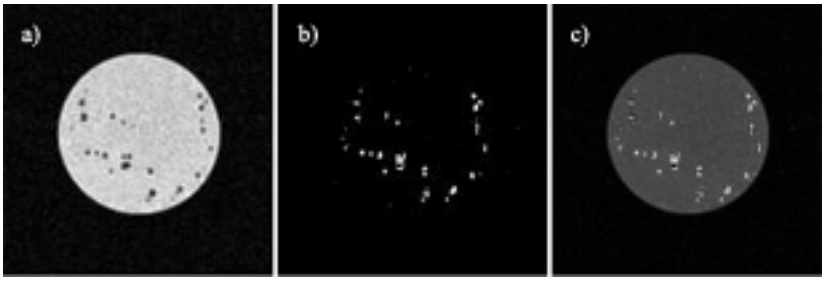

Fig. 1: Images from a single slice of SPIO-loaded cells suspended in gel using a) SPGR b) WMk SPGR c) Manual overlay of the two

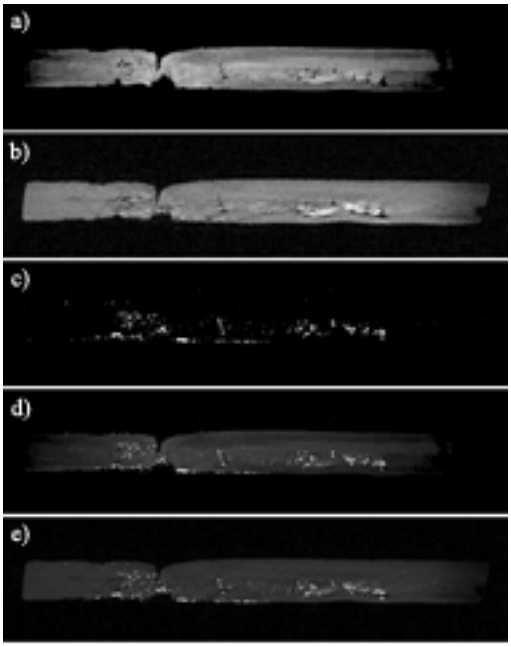

Fig. 2: Single MR slice of rat spinal cord injury using a) FIESTA b) SPGR c) WMk SPGR d) WMk overlaid onto FIESTA e) WMk overlaid onto SPGR

Conclusion

The white-marker sequence provides a novel approach to cellular imaging, which may also provide a method of separating the signal loss contributions of weak field perturbers, such as hemorrhage, air bubbles, or surface boundaries, from those of strong perturbers, such as iron-loaded single cells.

References and Acknowledgements

1. Seppenwoolde *et al*, MRM 50:784-790 (2003). 2. Foster-Gareau *et al*, MRM 49:968-571 (2003). 3. Bowen *et al*, MRM 48:52-61 (2002).

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