Detection and Quantification of Magnetically Labeled Single Cells in Live Animals by MRI

Erik M Shapiro[1], Ronen Globinsky[2], Xenios Papademetris[2], and Margaret F Bennewitz[3]

[1]*Department of Radiology, Michigan State University, East Lansing, Michigan, United States,* [2]*Department of Radiology, Yale University School of Medicine, New Haven, Connecticut, United States,* [3]*Department of Biomedical Engineering, Yale University, New Haven, Connecticut, United States*

INTRODUCTION: Cell transplantation has been implemented for a variety of purposes including tissue regeneration, delivery of therapeutic agents, and elucidation of biological processes. To evaluate their distribution *in vivo*, transplanted cells need to be tracked. For clinical applications, noninvasive imaging is a more practical alternative to histology, as experiments are performed longitudinally on intact, complex organisms, without the need for surgery. Compared to other noninvasive imaging modalities, MRI is ideally suited for cell tracking, due to its high spatial resolution, exceptional soft tissue contrast, excellent depth of penetration, ability to acquire 3-D images quickly, and lack of radiation. While the detection of single cells was first demonstrated in 2006 (1-3), quantification schemes for single cell MRI are scarce. In this work, MPIO labeled single MSCs were detected in vivo with 3D T_2* MRI and quantified using automated image processing techniques. The presence of magnetically labeled single cells in the brain was confirmed with histology.

MATERIALS AND METHODS:

Cell labeling with MPIOs: Primary rat MSCs were labeled in culture with Suncoast Yellow MPIOs (orange fluorescent) at 40 beads/cell, and CFSE, which labels the cytoplasm of live cells green.

Single cell in vitro agarose samples: MSCs with and without MPIOs were suspended in 0.5% agarose containing 1 mM gadoteridol at 100,000 cells/ml.

Single cell model in the rat brain: A model of single, dispersed cells in the rat brain was created by intracardiac injection of either magnetically labeled, CFSE labeled MSCs or non-magnetic cells. Adult Fischer rats were anesthetized and 200,000 MPIO labeled MSCs or unlabeled MSCs (200 µL of 1 x 10^6 cells/ml) were injected slowly with into the left cardiac ventricle. Animals remained under anesthesia for 1 – 1.5 hours before being imaged at 11.7T. Immediately following MRI, rats were sacrificed and brains were prepared for frozen sectioning at 16 µm thick sections. Brain sections were analyzed for green CFSE stained MSCs with red MPIOs.

Magnetic Resonance Imaging: MRI of the in vitro agarose samples was performed at 4.0 T. 3D T_2* gradient echo images were acquired at 50 μm^3 and 100 μm^3 isotropic resolution with TR = 100 ms, TE = 5, 10, 15, 20, 25 ms. MRI of live rats was performed on an 11.7T Varian system. T_2* weighted 3D gradient echo MRI was performed at image resolution = 100 x 100 x 133.3 µm, 2 different TRs = 30 ms and 60 ms, 3 different TEs = 8, 12, 15 ms.

Automated Spot Detection and Quantification: Automated spot detection and quantification was accomplished using similar techniques used for automated vessel detection (4). The 3D T_2* raw MRI data was first prepared for analysis through either being cropped to remove the signal drop-off artifacts at the edges of the scans (in vitro agarose data) or segmented to isolate the brain tissue from surrounding structures such as the skull (in vivo data). Next, the Hessian matrix, or second derivative, and the associated Eigenvalues were calculated from the raw data. The filter then selected for shape using the following process: 1) Eigenvalues were sorted from smallest to largest for each voxel, and the smallest Eigenvalue was selected for every voxel. 2) To isolate spheres (the signal voids of iron labeled cells are roughly spherical), the largest values within this smallest set of Eigenvalues were selected as positive responses, and reflected as such in the newly formed 2D Hessian images. Following sphere detection, the resulting Hessian images were thresholded to form 2D binary spot images, according to the image histogram, which served to isolate the original dark spots in the raw MRI data. Spots were quantified from the resulting binary images through cluster thresholds. A number was assigned to each spot, and the center of mass (i, j, and k coordinates) as well as the volume in voxels, was determined for each spot.

RESULTS and DISCUSSION: The average iron content within each labeled cell (76% of the cells are labeled) was 14 ± 0. 4 pg, or about 13 MPIOs. Figure 1 shows heterogeneous cell labeling; therefore, signal voids with various sizes and intensities will be created on MRI. Magnetically labeled single cells suspended in agarose produce distinct, robust dark spots on T_2* weighted MRI; each hypointense spot in Figure 2 represents a single MSC labeled with multiple 1.63 µm MPIOs. A characteristic 2D version of the raw MRI data and the corresponding output binary spot image, are also shown in Figure 2. In this methodology, Hessian Eignenvalues are used to select for spherical shapes, while thresholding allows for intensity selection to isolate the hypointense spots. An important feature of the filter is its ability to detect spots of different sizes, which correspond to cells labeled with various amounts of MPIOs. Detected spots showed a distribution in the amounts of voxels making up their volumes. In addition, through comparison of the 2D versions of the input and output, shown in Figure 2, it is clear that the filter identifies nearly all of the spots present in the input image. A 3D output reconstruction image is shown, with all of the detected signal voids inside the agarose sample. Using clustering thresholds, the program quantified the number of spots within the sample to be a total of 1,400 signal voids, or MPIO labeled MSCs.

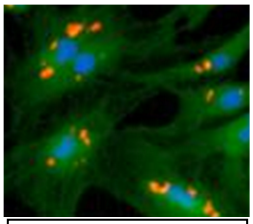

Figure 1: MPIO (orange) and CFSE (green) labeled MSCs.

Figure 3 shows coronal, axial, and sagittal 2D T_2* MRI slices of rats injected with MPIO labeled MSCs. Unlabeled control cells did not cause a decrease in signal intensity, whereas rats injected with magnetically labeled MSCs exhibited numerous dispersed, dark spots throughout the brain. These dark spots were well distributed throughout the whole brain along the rostral-caudal and superior-inferior axes. Different numbers of MPIO labeled MSCs, ranging from 1 to 3 cells, were detected within imaging windows of 300 µm wide (which is 3x larger than a voxel on in vivo high resolution MRI). Out of 70 MSC identifications in brain histology slices, 67% comprised 1 labeled cell, 26% comprised 2 labeled cells, and 7% comprised 3 labeled cells. MPIO labeled MSCs appeared to be inside blood vessels.

The spot counts in the brain for low and high thresholds were: 7,765 signal voids were detected for the low threshold and 1,504 were detected at the higher one. This means that the true number of labeled MSCs in the brain after intracardiac injection is between 0.8% to 3.9% of the total number of injected cells. To alleviate this thresholding issue, the easiest solution is to further optimize MPIO labeling, which in turn will enhance dark contrast.

NIH grant: DP2 OD00436 **Refs**: **1)** Shapiro, et al, MRM 2006; **2)** Heyn, et al, MRM 2006; **3)** Wu, et al, PNAS 2006; **4)** Joshi, et al, IEEE Trans. Vis. Comput. Graph.

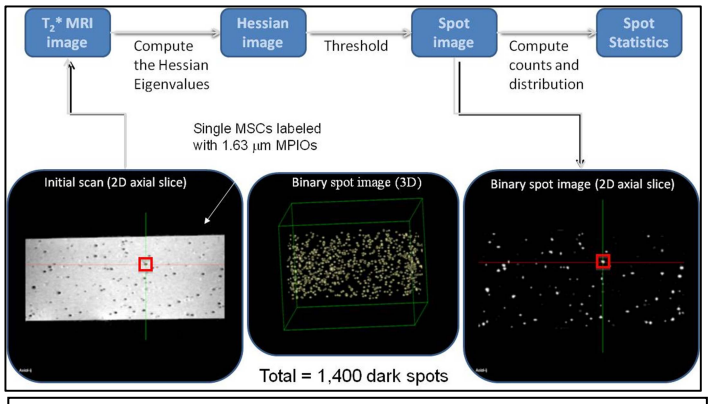

Figure 2: Automated sphere detection and quantification *in vitro* using image processing. a) 2D sagittal MRI slice of a 3D T2* gradient echo scan of MPIO labeled cells in agarose. b) 3D compilation of detected spots (i.e. magnetically labeled cells) within the agarose sample. c) Corresponding spots identified by the automated sphere detection program, based on intensity and geometry. The output image shown here is a binary spot image.

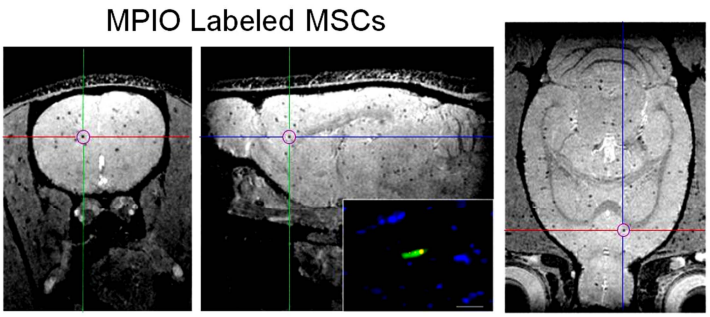

Figure 3: Detection of magnetically labeled single cells in the rat brain in vivo. Purple circles show a single spot in all three orthogonal planes, confirming the susceptibility effects induced by the labeled cells are spherical at this acquired resolution. Inset is a single MSC in green, MPIOs in orange, nuclei in blue.