In-vivo tracking of single phagocytic cells in a mouse brain after traumatic brain injury using micron-sized iron-oxide particles

T. K. Hitchens[1,2], P. H. Mills[1,2], L. M. Foley[1], J. A. Melick[3], P. M. Kochanek[3,4], E. T. Ahrens[1,2], and C. Ho[1,2]

[1]Pittsburgh NMR Center for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, United States, [3]Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA, United States, [4]Department of Critical Care Medicine and Anesthesiology, University of Pittsburgh, Pittsburgh, PA, United States

Introduction

The ability to non-invasively detect the trafficking and accumulation of cells *in vivo* has broad implications for a better understanding of biological processes and the development of novel treatments for numerous conditions. Previously, we applied cellular imaging techniques to quantify macrophage accumulation in a mouse model of Traumatic Brain Injury (TBI) [1]. Macrophage were labeled *in situ* by direct *i.v.* injection of micron-sized paramagnetic iron-oxide (MPIO) particles and the MPIO-labeled cells were detected as discrete hypointense spots using high-resolution magnetic resonance microscopy (MRM) on excised brains. With this method, single cells were detected throughout the brain, and accumulating at the site of trauma. Detecting and tracking single MPIO-labeled cells *in vivo,* however, presents a challenge due to scan time limitations when imaging live animals. Limited scan time leads to lowered image resolution and/or signal-to-noise ratio, which are both important for achieving adequate image contrast from each labeled cell. In addition, intrinsic sources of contrast in the brain may confound unambiguous detection of single MPIO labeled cells *in vivo*.

Here we explore serial tracking of single MPIO-labeled cells in the mouse brain *in vivo* using high-resolution 3D imaging and a post-processing method called Phase map cross-correlation Detection and Quantification (PDQ) [2]. PDQ works with conventional T_2^*-weighted images, using phase information to automatically identify magnetically-labeled cells throughout an MRI volume, and measure their magnetic dipole moment [3]. With this technique, magnetically labeled cells can more easily be correlated between serial scans and imaging methods.

Methods

Male C57Black/6J mice aged between 11-15 weeks were used for these studies. Mice were anesthetized with isoflurane in $N_2O:O_2$ (1:1), intubated, and mechanically ventilated; a femoral venous catheter was then surgically placed for MPIO injection (4.5 mg[Fe]/kg). Mice were injected with 0.96 µm MPIO (Bangs Labs, Fishers, IN). The mouse controlled cortical impact (CCI) model was used as previously described [1]. Mice were imaged at different time points using a Bruker 7-Tesla/21-cm AVANCE AV3 scanner equipped with a 35 mm mouse birdcage coil or a 2-cm T/R surface coil. High resolution T_2^*-weighted 3D images were obtained with the following parameters: TE/TR=7/100 ms, Averages = 4, and a resolution of 70×95×95 µm (volume coil) or 58×79×79 µm (surface coil). After the final MRI session, brains were perfused, fixed with 4% paraformaldehyde and imaged with MRM at 11.7-T with a resolution of 58×39×39 µm, as described previously [1].

Raw k-space data were processed to extract phase angle images, and phase-unwrapping algorithms applied to remove discontinuities at the [0, 2π] phase boundaries. PDQ was then used to search the phase data for 3D phase-offsets that matched a theoretical "dipole template" pattern, which approximates a sphere of magnetically-susceptible material. After each dipole was detected and its susceptibility (χ) measured using a least-squares fit, its magnetic dipole moment (*m*) was calculated using:

$$m = 10^{19} \cdot B_o \frac{r^3}{3}\left(\frac{\chi}{1+\chi}\right)$$

where *m* is in units of pA·m^2, B_0 is the applied magnetic field, *r* is the radius of the magnetic deposit (labeled cell), and χ is the magnetic susceptibility of the cell. Dipole moments were calculated assuming r = 5 µm for each MPIO-labeled cell.

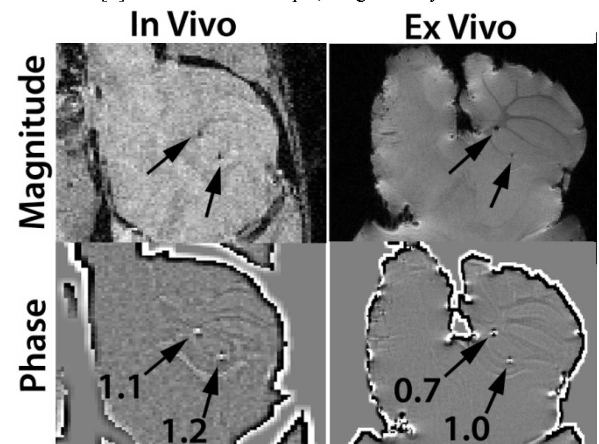

FIG. 1. Processed phase image from PDQ detection. **(Left)** *In vivo* detection of two MPIO-labeled cells with measured *m* values of 1.1 and 1.2 pA·m^2. **(Right)** Same two cells detected *ex vivo,* with measured *m* values of 0.7 and 1.0 pA·m^2, respectively.

Results and Discussion

Figure 1 shows how the PDQ technique was able to detect MPIO-labeled cells in both *ex vivo* and *in vivo* collected brain images. The calculated dipole moments for the MPIO-labeled cells were widely distributed presumably due to different numbers of MPIO particles loaded in each cell. For *ex vivo* detection, the average dipole moment was determined to be 0.4 ± 0.35 pA·m^2. For comparison of labeled cell detection between *ex vivo* and *in vivo* scans, brains were perfused and fixed immediately following completion of the *in vivo* scan. Preliminary results show that only a subset of cells detected *ex vivo* at 11.7-T, are found *in vivo* at 7-T (< 25%). The cells detected *in vivo* correspond to those with larger magnetic dipole moments, and more labeled cells were detected in the higher resolution scans. For example, the average magnetic dipole moment for cells imaged with a resolution of 70×95×95 µm is 1.4 ± 0.6 pA·m^2, where as the average is improved to 0.6 ± 0.3 (with more cells detected) at a resolution of 58×79×79 µm. Since PDQ provides a measure of the magnetic dipole moment, this information can be used to magnetically identify labeled cells at different time points in serial studies (Figure 2).

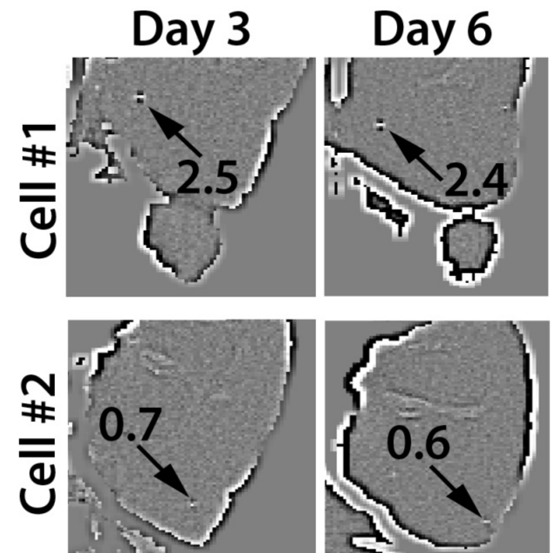

FIG. 2. Examples of serial detection of MPIO-labeled macrophages. Two cells are shown **(Rows)**. Each cell was detected in approximately the same anatomical location 3 days **(Left)** and 6 days **(Right)** following MPIO injection. For the first cell (**Top Row**) PDQ measured *m* values of 2.5 and 2.4 pA·m^2. For the second cell (**Bottom Row**) PDQ measured *m* values of 0.7 and 0.6 pA·m^2. Measured *m* values appear consistent over different scans and different time points.

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References

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