MRI-based cell tracking of OATP-expressing cell transplants by pre-labeling with Gd-EOB-DTPA

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Abstract: MRI-based cell tracking can play a key role in clinical cell therapies and is most useful when the high-resolution and spatial discrimination capabilities of MRI are used for determining the precise locations of transplanted cells. Traditionally, iron oxide nanoparticles are used for magnetic cell labeling with detection of labeled cells via T2/T2*-weighted MRI, however, the dark contrast obscures the underlying anatomy, and making quantification of cell number difficult. Bright contrast methods have been sparingly used for MRI-based cell tracking employing Gd-chelates and Mn^{2+}, accumulated intracellularly by disruptive (photoporation, transfection, etc) methods or by simple incubation, respectively. Further, ^{19}F agents have been used for MRI-based cell tracking. Yet, MRI-based cell tracking has not made significant clinical impact, partly due to the complications of cell labeling and detection.

Hepatic organic anion transporting polypeptides (**OATPs**) transport off-the-shelf, FDA-approved, hepatospecific Gd-based MRI contrast agents into cells that express the transporters, the intracellular accumulation of which cells causes signal enhancement on clinically familiar T1-weighted MRI. We show that OATP-expressing cells can be pre-labeled with Gd-EOB-DTPA prior to injection affording the use of clinically familiar T1-weighted MRI to robustly detect cell transplants. This straightforward approach to labeling and MRI detection may facilitate the incorporation of MRI-based cell tracking in clinical trials and cell therapies.

Introduction: MRI-based cell tracking can play a key role in clinical cell therapies and is most useful when the high-resolution and spatial discrimination capabilities of MRI are used for determining the precise locations of transplanted cells. Traditionally, iron oxide nanoparticles are used for magnetic cell labeling with detection of labeled cells via T2/T2*-weighted MRI[1], however, the dark contrast obscures the underlying anatomy, and making quantification of cell number difficult. Bright contrast methods have been sparingly used for MRI-based cell tracking (excellent review in [2]) employing Gd-chelates and Mn^{2+}, accumulated intracellularly by disruptive (photoporation, transfection, etc) methods or by simple incubation, respectively. Further, ^{19}F agents have been used for MRI-based cell tracking[3]. Yet, MRI-based cell tracking has not made significant clinical impact, partly due to the complications of cell labeling and detection.

Hepatic organic anion transporting polypeptides (**OATPs**) transport off-the-shelf, FDA-approved, hepatospecific Gd-based MRI contrast agents into cells that express the transporters, the intracellular accumulation of which cells causes signal enhancement on clinically familiar T1-weighted MRI[4]. We show that OATP-expressing cells can be pre-labeled with Gd-EOB-DTPA prior to injection affording the use of clinically familiar T1-weighted MRI to robustly detect cell transplants. This straightforward approach to labeling and MRI detection may facilitate the incorporation of MRI-based cell tracking in clinical trials and cell therapies.

Materials and methods: Lentivirus (Vectorbuilder) was used for stable transduction of human OATP1B3 in mammalian cells. HEK293 cells were infected at MOI 10:1. Following 3-week antibiotic selection and expansion, cells were labeled in media with 5.0 mM Gd-EOB-DTPA and 2 µg/ml indocyanine green (**ICG**) (both OATP1B3 substrates) for 1.5 hours, after which cells were washed. An aliquot of cells was pelleted and T1 was measured at 7.0T (Bruker Biospec). ICG cellular uptake was validated using fluorescence microscopy (Biotek Cytation3).

Following MRI validation of Gd-EOB-DTPA cell labeling, ~$5x10^6$ cells were pelleted and resuspended in 50 µl PBS. Food-grade chicken hearts were used as a model. ~$1x10^6$ cells in 10 µl PBS was slowly injected free-hand into the left ventricular wall of chicken heart (n=5) using Hamilton syringe and 22 gauge needle. Injected hearts were immediately imaged at 7.0T by 3D T1-weighted gradient echo MRI with parameters: TR/TE: 30/2.5, 1 average, FA 60°, resolution 250 um, FOV 40x30x30 mm, 8 min acquisition.

Images were analyzed in PMOD. 3D volumes of interest (**VOIs**) were drawn in non-injected heart region, region outside the heart (noise) and the hyperintense area from the injected cells. Contrast-to-noise ratio (**CNR**) was calculated.

After MRI, hearts were processed for cryofluorescence tomography (**CFT**) (Xerra, EMIT). Hearts were frozen over dry ice then embedded vertically in OCT in a 7.5x9.5x5 cm mold. White light and fluorescence images (excitation 780 nm, emission 835 nm) were acquired with 30 um in-plane resolution and 50 um slice thickness. Image stacks were combined in PMOD and maximum intensity projections of the fluorescence images were computed.

Results and discussion: Stable OATP1B3 expression in HEK293 cells was confirmed by qPCR and phenotypically by uptake of Gd-EOB-DTPA and ICG. T1 time for OATP1B3-expressing cells were 52 ms following incubation, while non-expressing cells was ~1500 ms. ICG uptake into cells was verified microscopically and is an important marker for validating cell location in vitro. In the hearts, the calculated volume of cells generated from MRI VOIs was 6.7 +/- 2.3 µl, very close to the 10 µl injected volume, with CNR 38.4 +/- 15.4. CFT validated near identical location of injected cells, verifying that the bright MRI signal was generated from the ICG-labeled transplanted cells.

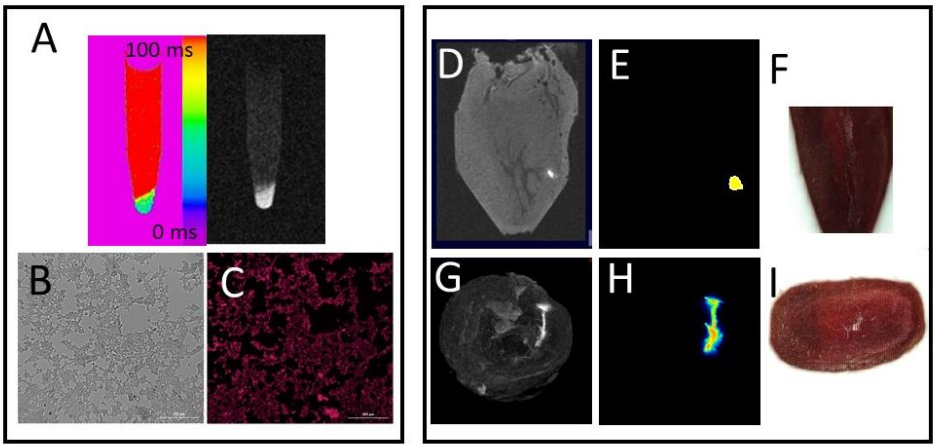

Figure 1: A) T1 map and corresponding MRI of OATP1B3-expressing cells following incubation in 5.0 mM Gd-EOB-DTPA and 2.0 µg/ml ICG for 1.5 hours. B,C) White light (B) and NIR fluorescent image of OATP1B3-expressing cells harboring intracellular ICG following labeling. D,E) MRI of chicken heart with bright contrast spot from Gd-EOB-DTPA labeled OATP-expressing cells (D) confirmed by ICG NIR fluorescence from CFT image (E). F) White light image corresponding to area in (E). G, H) Maximum intensity projection image from MRI of entire heart showing bright contrast from Gd-EOB-DTPA labeled OATP-expressing cells (G) confirmed by ICG NIR fluorescence from CFT image (H). I) White light image corresponding to area in (H).

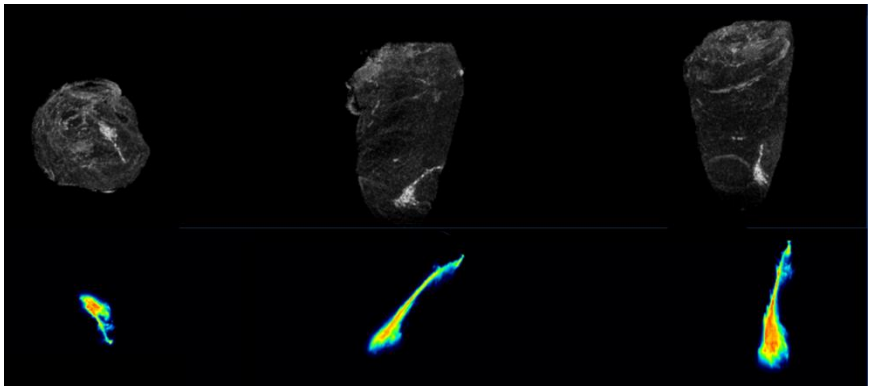

Figure 2: Second Example of MRI-based cell tracking of Gd-EOB-DTPA labeled OATP-expressing HEK293 cells transplanted into chicken hearts. Maximum intensity projections from MRI in orthogonal views **(Top)** with corresponding maximum intensity projections from CFT showing corroborating positions.

Conclusion: MRI-based cell tracking of OATP-expressing cells following transplantation is robust and feasible by pre-labeling cells with Gd-EOB-DTPA.

References:

1. Slotkin, J. R.; Cahill, K. S.; Tharin, S. A.; Shapiro, E. M., Cellular magnetic resonance imaging: nanometer and micrometer size particles for noninvasive cell localization. *Neurotherapeutics* **2007,** *4* (3), 428-433.
2. Cheng, H.-L. M., A primer on in vivo cell tracking using MRI. *Frontiers in Medicine* **2023,** *10*.
3. Ahrens, E. T.; Bulte, J. W., Tracking immune cells in vivo using magnetic resonance imaging. *Nat. Rev. Immunol* **2013,** *13* (10), 755-763.
4. Patrick, P. S.; Hammersley, J.; Loizou, L.; Kettunen, M. I.; Rodrigues, T. B.; Hu, D. E.; Tee, S. S.; Hesketh, R.; Lyons, S. K.; Soloviev, D.; Lewis, D. Y.; Aime, S.; Fulton, S. M.; Brindle, K. M., Dual-modality gene reporter for in vivo imaging. *Proc Natl Acad Sci U S A* **2014,** *111* (1), 415-20.