IN VIVO CELL TRACKING BY ^{19}F MRI USING PERFLUOROCROWN ETHER NANOPARTICLES

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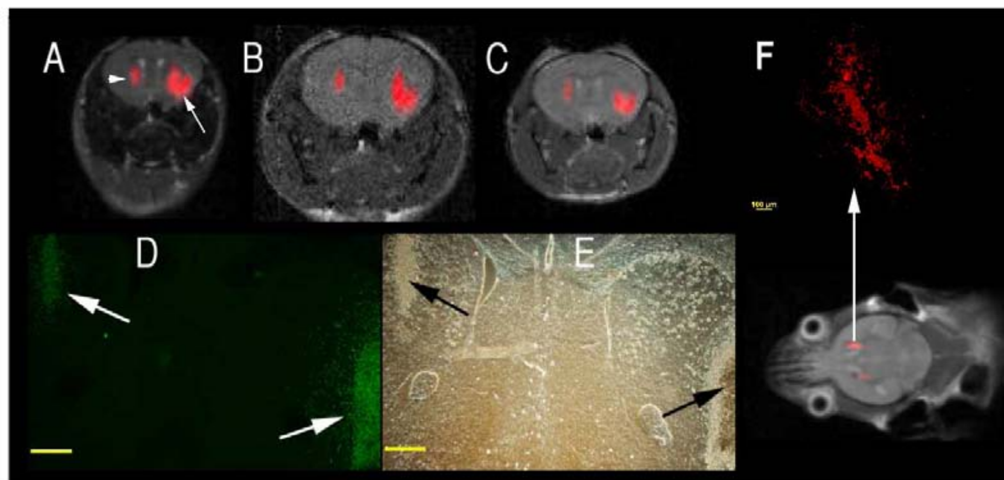
INTRODUCTION: Magnetic resonance imaging (MRI) allows visualization of labeled cells *in vivo* in real time, and has provided new insights into the biodynamics of cell trafficking and migration. Fluorine labeling has emerged as a new alternative method for MRI cell tracking (1). Cells are incubated with the fluorine agent (i.e., an emulsion of fluorocarbons) *in vitro* in order to pre-label cells before administration. ^{19}F-MRI has unique imaging features for evaluation of cell trafficking and migration. The fluorine atom is 100% naturally abundant, its NMR sensitivity is comparable to that of protons (around 0.86), with a negligible ^{19}F background signal (2). PFCE has a large number chemically equivalent fluorine atoms, with the ^{19}F spectrum as a single narrow resonance (avoiding chemical shift artifacts), making it an ideal ^{19}F tracer.

METHODS: C17.2 mouse neural stem cells were grown on Petri dishes coated with both carboxylic acid and amino groups. A cationic emulsion (+62.8 mV zeta potential) was formulated with 20% PFCE, and a mixture of lipids, including 15% w/v phosphatidylcholine (PC), 5% w/v cholesterol, 59.9% w/v 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), 20% w/v 1,2-dioleoylphosphatidylphosphoethanolamine (DOPE), and 0.1% w/v rhodamine. Cells were incubated with 2.4 or 4.8 mM final PFCE for 18 h, washed three times, and suspended in PBS at 40,000 cells/µL. Two C15/BL6 20 g male mice were anesthetized with ketamine/xylazine and were injected slowly into the striatum of both hemispheres ($4x10^4$ cells at one side, $3x10^5$ cells at the other side). Longitudinal ^{1}H and ^{19}F MRI studies were performed on a 9.4 T Bruker Biospec spectrometer (Bruker Biospin MRI, Billerica, MA, USA), using a custom-built slotted tube RF resonator tunable between ^{1}H and ^{19}F frequencies, up to 2 weeks after injections. ^{19}F MR images were obtained using a multi-slice (10 x 1 mm slices) fast spin echo sequence with TE=47 ms; TR=1079 ms; NA = 64; FOV=2.5x2.5 cm or 2.0x3.0 cm, and matrix=64x32. These were overlaid with high resolution ^{1}H MRI obtained with a standard multi-slice spin echo sequence (TR/TE 1000/15 ms, matrix 128x128, ST 1mm). The brains were excised, cryopreserved in 20% sucrose for 24h, cryosectioned at 20 µm, and processed for immunohistochemistry, fluorescence (red), and light microscopy. For immunohistochemistry, a mouse monoclonal antibody was used as the primary antibody against beta-galoctosidase and goat anti-mouse antibody as the secondary antibody.

RESULTS: The figure below shows ^{1}H MRI of a mouse brain injected with $4x10^4$ (arrow head in A) or $3x10^5$ (arrow in A) cells injected in each hemisphere, overlaid with the corresponding ^{19}F image. MRI was performed immediately (panel A), 3 days (panel B), and 7 days (panel C) after cell injection. The cell implants were immediately visible after injection (panel A) as a bright signal spot at the injection sites. One week after grafting (panel C), the signal remained unaltered with the same signal-to-noise ratio. Immunohistochemistry confirmed the presence of a large number of viable cells in both hemispheres (arrows in panels D, anti-gal-B immunohistochemistry, and E, phase contrast). Injected cells were still rhodamine-positive at two weeks (panel F, arrow), indicating retention of label for this period.



CONCLUSION: PFCE-labeled cells remain viable after two weeks following intrastriatal injection, without an appreciable reduction in the fluorine signal for at least up to 2 weeks.

References:

1) E. Ahrens et al., Nat. Biotech. 2005, 23: 983-987;
2) JWM Bulte et al., Nat. Biotech. 2005, 23: 945-946.