Positive contrast MRI of transplanted cells in rabbit quadriceps in vivo

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Introduction: Our overall research goal is the design of non-invasive MRI methodologies to measure transplanted cell number, volume of distribution, migration, engraftment, differentiation and apoptosis which can guide optimization of clinical strategies for therapeutic cell delivery. The positive-contrast method, which selectively images the resonances of water in the vicinity of magnetically-labeled cells, is a beneficial development since the MRI signal local to the cells of interest is retained [(1)] . One can now consider a more comprehensive characterization of the affected MR signal than what is feasible using negative contrast strategies which may obliterate local tissue signal [(2,3)] .

The positive contrast technique uses a narrow-bandwidth (+/- 500 Hz) excitation and refocusing pulse pair. The RF is applied in the absence of slice-selective gradients such that the current methodology is limited to 2D projections. The RF center frequency is offset from the global resonance frequency of tissue water by approximately +/- 1 kHz, to excite selectively the spatial regions of matching resonance frequencies in the dipolar magnetic field surrounding a 'sphere' of transplanted and magnetically labeled cells. The initial studies in ex vivo tissue demonstrated a correlation between the area of positive contrast and labeled cell number. This abstract reports a demonstration of positive contrast imaging of magnetically labeled cells in vivo. This advance is a first step towards correlation of the positive contrast signal characteristics with robust histological indices of transplanted cell fate in vivo.

Methods: Studies were conducted in two 4kg New Zealand White rabbits by use of procedures and protocols approved of by the Research Ethics Board of Sunnybrook and Women's College. The quadriceps was selected as the target while rat fibroblasts were selected for magnetic labeling and transplantation Magnetic labeling was initiated on the day prior to MR experimentation via addition of iron-fluorescent particles (IFPs, Bang's Laboratories) to up to 4 T75 confluent flasks of fibroblasts (10 μg IFP stock solution per ml of fibroblast growth media (DMEM + 10% fetal bovine serum + 2% penicillin/streptomycin)). The cells were returned to their incubator and allotted with 18 to 20 hours for IFP endocytosis. At the end of this period, the cells of each flask were rinsed three times with phosphate-buffered saline, trypsinized via addition of 2.5ml of trypsin, and centrifuged at 1000 rpm for 5 minutes followed by aspiration of fluid. The pellets were diluted with 110, 250, 500, and 1000 μl of media respectively. Cell number per sample was calculated via coulter counter as the total particle number between 10 and 20 μm.

Each rabbit was anaesthetized and its quadriceps muscles were shaven concurrent with the final stages of cell preparation. Each rabbit was then positioned prone in the head coil of a 1.5 Tesla GE Signa, with a level of elevation so that each quadriceps was accessible for needle insertion. Axially-aligned sites for cell injection on each side of the femur were demarcated using a black permanent marker. Fiducials (vitamin E capsules) were fixed to the skin surface superior and inferior to the injection sites to aid in slice prescription. Fiducials were located rapidly using a series of axial SPGR slices and magnetization-preparation T1-weighted spiral images. The orthogonal projection to an oblique slice through these fiducials was the output of positive-contrast imaging. Following localization, a continuous infusion of Gd-DTPA was initiated to shorten T1 in the vicinity of labeled cells and hopefully to sensitize signal intensities to viable cell density (0.25 mmol/kg bolus, followed by continuous infusion in saline at 0.005 mmol/kg/min). An hour was allotted for equilibration of tissue Gd-DTPA concentration, following which 100 μl suspensions of each aliquot were extracted into a 100 μl volume Hamilton precision syringe and injected into the target muscle locations. T1-weighted positive contrast projection images (256 x 128, 20cm FOV, TI = 500 ms, TR = 1000ms) were then acquired at variable resonance offsets (+/- 900 Hz). Data analysis consisted of manual ROI prescription at each injection site, including only pixels with a per voxel SNR greater than 10.

Results: At +900Hz off-resonance, rabbit #1 demonstrated areas of enhancement of 150, 100, 42, and 43 mm^2, corresponding to injected cell numbers of 600, 300, 160, and 60k, while rabbit #2 demonstrated areas of enhancement of 100, 48, and 52 mm^2, corresponding to injected cell numbers of 600, 200, and 150k. A similar trend of signal enhancement was observed at -900Hz.

Summary and Conclusions:. In this in vivo study we have demonstrated that this off-resonance technique can provide adequate positive contrast to measure relatively small numbers of delivered cells. Differentiation between injected cell numbers of greater than 100k seems feasible using the area of positive contrast, while the detection threshold appears to be much less than the 100k limit suggested by Hill. However, comparative studies between fibroblasts and bone-marrow-derived stromal cells remains to be performed. This technique may prove useful to measure cell distribution, migration and apoptosis.

References: 1) Cunningham et al, MRM, Submitted, 2004; 2) Hill et al Circulation, **108**, 1009, 2003; 3) Frank et al. Radiology, **228**, 480, 2003.

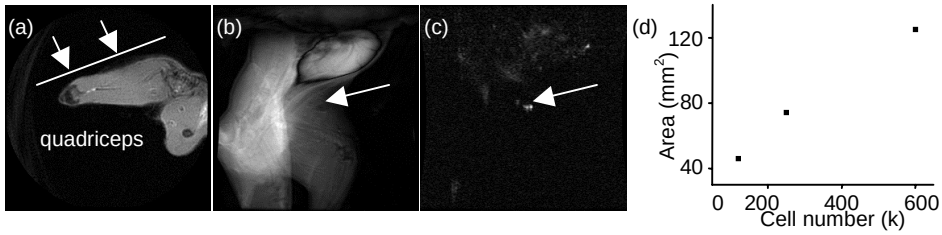

Figure 1: **(a)** The projection angle for +ve contrast imaging; **(b)** The -ve contrast projection through the left quadriceps. The arrow demarcates cell location; **(c)** The +ve contrast projection delineating a region of signal enhancement at the same location as in (b); and **(d)** +ve contrast area at mean cell numbers of 120k, 250k, and 600k, across both rabbits.