Tracking and quantification of T-cells labelled with iron oxide nanoparticles using positive contrast

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Purpose Cell-based therapies have received much attention as novel therapeutics for the treatment of cancer and autoimmune disease. To facilitate the clinical translation of cell-based therapies, noninvasive imaging will be needed to allow accurate and quantitative evaluation of cell biodistribution following injection. Super paramagnetic iron oxide nanoparticles (SPION) have been used for the non-invasive MRI tracking of cells with high sensitivity. Optical fluorescence imaging, PET, and SPECT have also been used to track labeled cells, although cell tracking with MRI has the advantages of avoiding ionizing radiation and the ability to image deep tissues at high resolution. However, in certain cases, the ability of MRI to quantify cell distributions in vivo is limited, since conventional T_2^*-weighted sequences lead to negative contrast and low signal-to-noise ratio (SNR). Due to the so-called "blooming effect", when SPION-labeled cells accumulate, the volume of the signal void seen in T_2^*-weighted images can greatly exceed the actual volume occupied by cells. SWIFT is a novel ultra-fast sequence that exploits frequency-swept excitation and a simultaneous signal acquisition strategy to capture signals arising from spins that decay very fast (1,2). While conventional MRI produces only negative contrast with increasing SPION concentrations, SWIFT produces positive contrast and translates this to high SNR in the concentration range of interest that can also be quantified (3-5). In this study, T-cells were labelled with SPIONs and quantified using the SWIFT Look-Locker T_1 mapping method (4).

Methods Five sets of primary T-cells with same number of cells (5 million donor derived peripheral blood mononuclear cells per well in RPMI media supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 µg/mL penicillin, and 100 µg/mL streptomycin) were incubated at 37 °C/ 5% CO_2 in SPIONs (Ferrotec, NH, USA) solutions at concentrations of 0, 0.005, 0.01, 0.05, 0.1 mg Fe/ml with 3% DMSO for 30 minutes (6). The cells were then washed two times with PBS, re-suspended in PBS, transferred into NMR tubes with pre-solidified agar and centrifuged to pellet the cells. After removing the supernatant, cells were trapped with a second layer of agar. The MRI images and SWIFT T_1 maps were acquired on a 9.4 T animal MRI scanner (Agilent Technologies, USA) using a home-made three loops coil, with BW=384 kHz, TR=1.2 ms. The SWIFT T_1 map was acquired by SWIFT Look-Locker method with 4096 views in one saturation-recovery cycle and 32 sets of views (4). GRE images were acquired also for comparison using BW=150 kHz, TR=4.2 ms, TE=2.1 ms. TEM images of the SPION labeled T-cells were also acquired.

Results and discussions MR images and T_1 maps of cell-agar phantoms with T-cells incubated at different concentrations of SPIONs are shown in Fig. 1a. It is apparent that SWFT images have very high SNR while the standard T_2^*-weighted GRE images are approximately at noise level (Fig. 1b). The R_1 (=1/T_1) measured by SWIFT is linearly dependent on the concentration of SPIONs used in cell labelling (Fig. 1c). Here we assume the concentration of SPIONs in the cells is proportional to that used for labelling. The TEM data (Fig. 1d&e) demonstrated the T-cells can be loaded with non-toxic amounts of SPIONs under mild conditions in the presence of a small percentage of DMSO. It can be seen that the SPIONs are well dispersed (not aggregated) in the vesicle of the cell. Several (not all) piles of IONPs are indicated with blue arrows.

Conclusions This study shows for the first time that SPIONs in T-cells can be quantified with T_1 positive contrast. The longitudinal relaxation rate constant (R_1=1/T_1) measured by SWIFT sequence showed a linear dependence on the concentration of SPIONs in T-cells. The preliminary results indicate a potential role for positive contrast from SWIFT as a means to quantify the distribution and density of SPION-labeled T-cells, provided the cells are incubated at a constant SPION concentration.

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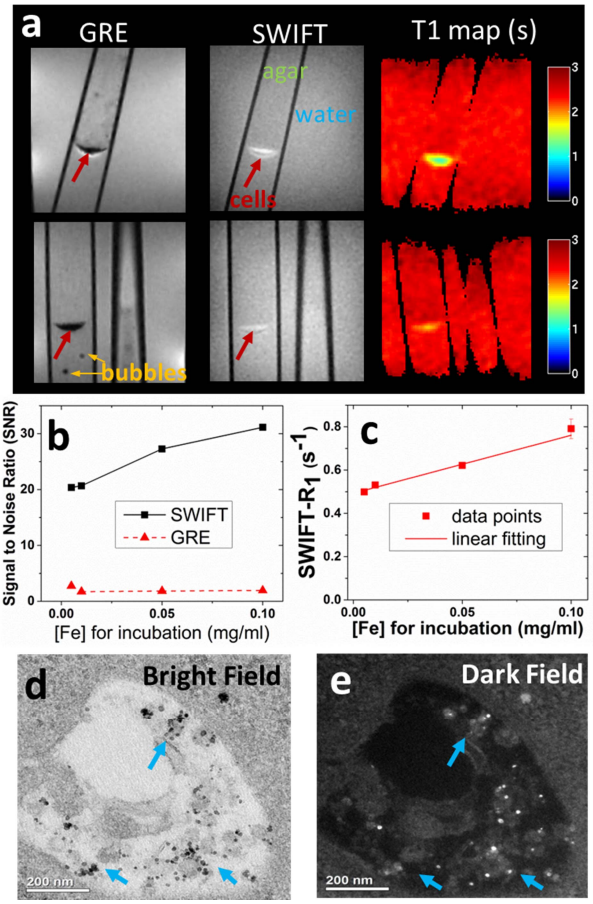

Figure 1. (a) MR images and maps of T-cells (red arrows) labeled with SPIONs at different iron concentration ([Fe]). (b) SNR of the cell regions from SWIFT and GRE images. (c) Plot of the SWIFT R_1(=1/T_1) relaxation rate versus incubation concentrations. A linear relationship was observed. (d) and (e) are TEM images of a vesicle in one T-cell with bright field and dark field. Several (not all) SPIONs were indicated with blue arrows (dark spots in d) and bright spots in e)).