Single Cell Detection with FIESTA: Effect of Iron Loading and Distribution

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Synopsis
Detection of single SPIO-labeled cells is possible using the SSFP sequence FIESTA (Fast Imaging Employing Steady State Acquisition). Little is known, however, about the effects of Fe mass and distribution on contrast. Utilizing a methodology for MR imaging and optical verification of single or multiple cells, we investigated the effect of Fe mass and distribution on susceptibility-induced contrast with FIESTA. We determined the lower limit of Fe detection to be between 24.5fmol and 54fmol for a 100x100x200µm voxel. CNR increases approximately linearly with increasing Fe/voxel, however, Fe mass/cell appears to also be important in determining contrast with FIESTA.

Introduction
Over the past decade, Superparamagnetic Iron Oxide (SPIO) has gained widespread use as an MR contrast agent for imaging stem cell and immune cell migration. We have previously demonstrated that FIESTA can be used to detect single SPIO labeled cells, ex vivo, at 1.5T. In the present work, we attempt to determine the effect of SPIO mass and distribution on susceptibility-induced contrast using the FIESTA pulse sequence.

Methods
**Cell Culture:** THP-1 cells, a human macrophage cell line, were labeled with SPIO nanoparticles (SHU 555A, Schering AG) and fluorescently labeled with a lipohilic carbocyanine dye DiI (1,1-di-octadecyl-3,3,3',3'-tetramethyl-lindocarbocyanine perchlorate). SPIO uptake into THP-1 cells was varied by incubating at different SPIO concentrations (48, 12, 3µgFe/ml) for 24 hours. To assess cellular Fe uptake, Atomic Emission Spectroscopy (AES) was performed. To facilitate MR and optical imaging, cells were placed in a single plane sandwiched between two layers of 8% w/w gelatin in an optically transparent plastic micro-well (inner diameter 7mm).

**MR and Optical Imaging:** MRI of cells was performed on a 1.5T GE CV/i MR using a custom-built gradient coil (inner diameter 12cm, maximum gradient strength 600mT/m and peak slew rate 2000T/m/s) and a customized solenoidal radiofrequency coil (1cm diameter, 1cm length). Samples were scanned using 3DFIESTA (TR/TE 6.3/3.2ms, flip angle 50°, 100µm in plane resolution, 200µm slice thickness). Total acquisition time/sample was about 5 minutes. Optical imaging was performed on an Olympus IX50 inverted microscope equipped with epifluorescence and a Sony 3CCD color digital camera. 25 fluorescence and bright field photomicrographs of each cell sample were taken using a high-powered objective in order to distinguish between single cells and clusters of two to three cells. The complete field of view was reconstructed by overlapping the photomicrographs to produce a collage.

**Correlative MR and Optical Imaging:** For each cellular Fe loading, MRI cell patterns (created by SPIO induced signal loss and visualized as discrete “black holes” on FIESTA images) were overlaid onto fluorescent cell patterns from optical collages. For each discrete area of signal loss, corresponding to a SPIO labeled cell or multiple cells, the contrast-to-noise ratio (CNR) was calculated for the central voxel relative to the background gel. CNR was correlated with the number of cells within the voxel, as determined from the optical images.

Results
Figure 1: Fe uptake into THP-1 cells was 1.37±0.09, 3.02±1.15, and 6.17±1.90pgFe for incubations with 3, 12, and 48µgFe/ml respectively. Cells labeled with 1.37pgFe/cell could not be detected in FIESTA images. Fluorescence micrograph of DiI labeled THP-1 cells (a) and corresponding FIESTA image (b). Graph of CNR for 1, 2 and 3 cells/voxel at two different intracellular Fe loadings (c) and the same graph of CNR, but normalized by the amount of Fe/voxel (d). CNR increases with increasing Fe mass/voxel. When the CNR data is normalized by the concentration of iron, there is a significant increase in normalized CNR for cells containing 6.2pgFe/cell compared to those containing 3pgFe/cell.

Conclusions
The minimum amount of iron needed within a 100x100x200µm (2nL) voxel for detection using the 3DFIESTA pulse sequence under our experimental conditions ranges between 1.37pg (24.5fmol) and 3.02pg (54fmol). The CNR for a voxel containing SPIO labeled cells increases with the amount of Fe within the voxel. Interestingly, when CNR is normalized by the mass of Fe/voxel, there is a significant difference in FIESTA contrast between cells containing different amounts of iron. This suggests that for the FIESTA pulse sequence, both iron concentration and the mass of Fe/cell play a role in generating susceptibility-induced contrast.

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