Sizing It Up: Efficient Endocytosis of Micron Sized Iron Oxide Particles for Cellular MR Imaging

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Introduction

Molecular and cellular MRI would be aided by robust single cell detection in live subjects. Cell labeling is most often accomplished by endocytosis of nanometer sized dextran coated iron oxide particles (USPIO's, MION's), yielding upwards of 50 pg of iron/cell (1). Present labeling strategies have accomplished single cell detection *in vitro* (2) and down to a few cells *in vivo* (3). The limitations of using USPIO's or MION's however, are that millions of particles are required to see single cells, the label can be diluted beyond observability by cell division, and the label is biodegradable. This makes *in vivo* labeling of cells other than macrophages, and long term engraftment studies difficult. Recently, it has demonstrated that some cells readily endocytose polymer encapsulated micron size iron oxide microparticles (MPIO's) (4). These particles have higher relaxivity than USPIO's based on equivalent iron content (4), with iron quantities of 0.1-1.6 pg iron/particle. Indeed, cells harboring just single 0.96 micron particles can be detected by MRI, even in complex tissues, so cell division cannot dilute the label from detection (5). We investigated the capacity of cells to endocytose three different size particles, 0.96, 1.63 and 2.79 microns, and measured their MRI signatures at three different field strengths, 4.7, 7.0 and 11.7 Tesla and with different imaging resolutions and echo times. These measurements will aid in the design of *in vivo* studies by determining necessary imaging conditions for single cell detection in various systems.

Materials and Methods

Mouse hepatocytes were isolated using standard methods (6). Hepatocytes were labeled with fluorescently impregnated MPIO's by simple overnight incubation of confluent cell culture dishes (10^6 cells) with 10^9 particles. Cell viability was evaluated with trypan blue exclusion tests. Free particles were removed by rigorous washing followed by Ficoll-Paque density centrifugation. Cells were plated on collagen coated glass coverslips at 50 cells/well for imaging. Growth medium was supplemented with Magnevist to enhance T_1 of the solution, allowing rapid imaging. Live cells were imaged at the three different field strengths using 3D gradient echo imaging with the following parameters: TR = 100 ms, TE = 3, 5, 10 or 20 ms, FOV of 5.12 x 2.00 x 0.64 cm. Image resolution was varied from 50 to 200 microns. Fluorescence and light stereomicroscopy were used to measure labeling efficiency and to count the number of particles per cell at each step of the labeling and washing procedures.

Results

Figure 1A-C shows 3 different cells labeled with (a) 0.96, (b) 1.63 and (c) 2.79 micron particles. Particles remain exclusively in the cytosplasm, and appear to be densely clustered in perinuclear endosomes. More than 100 particles can consistently be counted in cells labeled with the two smaller particles, while as many as 75 can be found in cells labeled with the 2.79 micron particles. This equates with ~ 10 to more than 100 pg of iron/cell. Labeling efficiency was near 100% in all batches. Following trypsinization to remove cells from culture flasks, fluorescence and light stereomicroscopy confirmed the continued presence of particles in cells, including after density centrifugation, and replating for imaging. Trypan blue exclusion tests revealed no enhanced cell death due to labeling.

Figure 2A-D shows images of four wells from a culture dish, with control cells, 0.96, 1.63 and 2.79 micron labeled cells from left to right. This image is at 100 x 50 x 50 microns resolution and acquired at 4.7 Tesla with an echo time of 10 ms. As can readily be seen, control cells elicit no contrast, while the labeled cells all elicit similarly sized, dark, susceptibility induced contrast. The size of the contrast region varied from 300 to 500 microns in diameter, independent of image resolution. At higher resolutions, the classic 'triangle shaped' susceptibility artifact can be visualized. Lower resolutions reduced the artifact shape to a more circular appearance. The size of the contrast region was independent of field strength. Shorter echo time images reduced the average contrast region ~ 25% for 5 ms TE and ~ 50% for 3 ms TE. A 20 ms TE increased the average contrast region nearly 25%.

Discussion

This work demonstrates that cells loaded with MPIO's of various sizes can produce contrast regions of several hundred microns in diameter. The contrast area is generally independent of field strength, yet the size of the contrast region can be increased by lengthening the echo time. Routine 3D gradient echo MRI can image a rodent brain in 1-2 hours at 100 micron resolution with good signal to noise. This is key, because were one to aim towards single cell detection in the brain of a live rodent, for example, a contrast region of at least 200 microns in necessary. It is therefore, our hypothesis that single cells loaded with MPIO's will be detectable *in vivo*, as their contrast area, at 100 microns resolution, would be nine square pixels in plane. Even at 200 microns resolution, the contrast area would encompass 4 square pixels. Additionally, because brain has a relatively long T_2^*, under well shimmed conditions, the echo time can be extended to at least 20 ms, to increase the size of the contrast region to 16 square pixels at 100 microns resolution. For visualizing single cells homing to tissues with shorter baseline T_2^*, such as bone marrow, liver or muscle, the echo time would have to be shortened in order to better observe those tissues. In this case, the minimal useful image resolution would be 100 to 150 microns, as the contrast size decreases with shorter echo time, or increase the SNR to better delineate the contrast region boundaries.

This work justifies further research into the development of novel particles, larger than 1 micron. A single 2.79 micron particle has a larger volume by ~ 25 times than a 0.96 micron particle. Fully maximizing the capacity of these larger particles with efficient iron loading would dramatically increase the susceptibility of such a particle, and hence its potency, making single particle detection inside single cells more robust, even at lower, clinically relevant image resolutions. Labeling cells with these particles may alleviate the stress of efficient labeling schemes, and perhaps permit *in vivo* labeling of cell populations. Continued work to determine the upper size limit for particle endocytosis by several cell types is under way.

References: 1) Weissleder, R, et al, *JMRI*, **7**:1, 258-63, (1997); 2) Dodd, SJ, et al, *Biophys J*, **76**, 103-9, (1999); 3) Kircher, MF, et al, *Cancer Res*, **63**:20, 6838-46, (2003); 4) Hinds, KA, et al, *Blood*, **102**:3, 867-72, (2003); 5) Shapiro, EM, et al, *Proc ISMRM 2003*, #229; 6) Seglen, PO, *J Toxicol Environ Health*, **5**, 551-60, (1979).

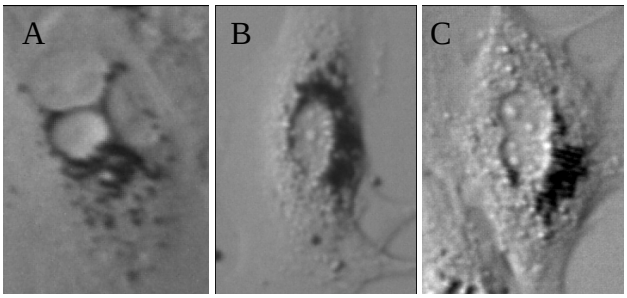

Figure 1: Hepatocytes labeled with A) 0.96, B) 1.63 and C) 2.79 micron particles. Dark perinuclear spheres are individual MPIO's. More than 100 0.96 and 1.63 micron particles and as many as 75 2.79 micron particles can be counted in cells.

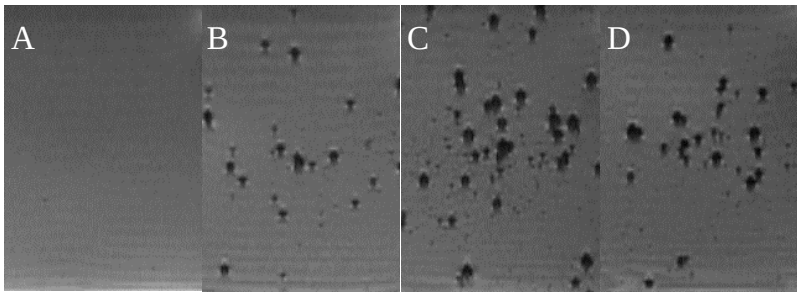

Figure 2: Four wells from a cell culture dish with A) unlabeled, B) 0.96, C) 1.63 and D) 2.79 micron particle labeled hepatocytes. Individual contrast regions are ~ 300 – 500 microns in diameter. Image is at 100 x 50 x 50 microns with a TE of 10 ms.