In-vitro and In-vivo Cellular Imaging Using Micrometer-Sized Iron Oxide Particles

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Introduction:

Solid organ transplantation is an established therapeutic procedure for treatment of patients suffering from end-stage nonmalignant organ failure. However, the long-term survival of organ recipients is threatened by repeated episodes of acute and the later development of chronic rejection, resulting in accumulative damage that may lead to graft failure or death. Magnetic resonance imaging (MRI) has been shown to be a powerful tool for non-invasive monitoring and evaluation of solid organ rejection if a suitable quantity of superparamagnetic contrast agent can be targeted to rejection sites. The purpose of this study is to demonstrate that micrometer-sized iron oxide particles (MPIOs) can be utilized for efficient labeling of immune cells, such as T-cells and macrophages, and that these labeled cells can subsequently be detected *in-vitro* and *in-vivo* using MRI. Since superparamagnetic contrast agents disrupt the magnetic susceptibility of water over an area 50 times the radii of the particles, the micron-sized iron oxide cores should provide enhanced *in-vivo* sensitivity. To evaluate the lower limit of *in- vivo* detection, MPIO labeled macrophages were infused into the kidney of a rodent transplant model and the threshold of *in-vivo* detection of labeled cells was investigated using MRI.

Methods:

Macrophages isolated from BN rats were labeled by incubation with 0.35, 0.90, and 1.63 μm fluorescence labeled Bangs particles. Labeled and control cells ($2.0 \times 10^3/\mu l$ in 1.0 % agarose gel) were imaged at 11.7-T. To evaluate *in-vivo* MRI response, the left kidney was isolated and then the left renal artery was ligated. After flushing out the blood from the left kidney, the left renal vein was then ligated. In-vivo MRI was performed before and after infusion of 2.4×10^6 MPIO labeled macrophages via left renal artery.

Results:

The multiple particle uptake of 0.90 μm MPIOs by macrophages is evidenced by the representative transmission electron micrograph (TEM) in Figure 1. Nearly 100% of macrophages cultured in the presence of label incorporate one or more of the micron-sized particles (TEM and fluorescence microscopy results). Labeled cells suspended in gel were readily detected at a concentration of 2.0×10^3 cells/μl using MRI (11.7-T) as shown in Figure 2. Each dark area observed in the image is believed to represent a single cell containing one or more particles and the characteristic barbell shaped negative contrast is observed.

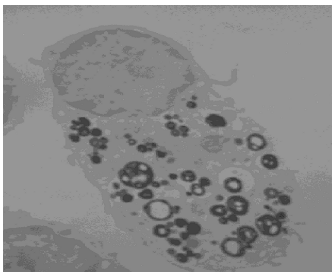

Figure 1

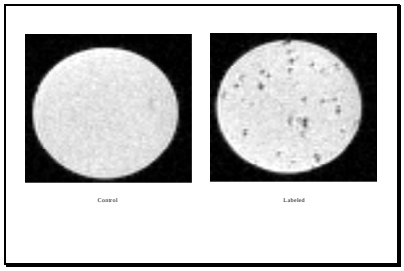

Figure 2

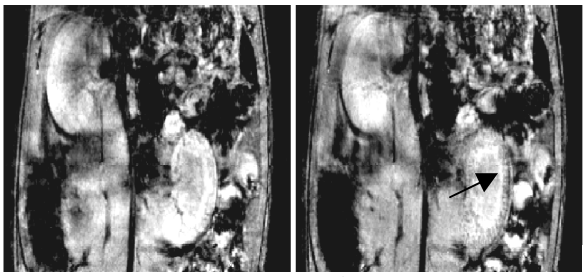

Figure 3

To evaluate the number of adoptively transferred labeled cells required for detection in the organs of rodent models, 2.4×10^6 macrophages, labeled with 0.90 μm particles in 800 μl PBS were injected into the left kidney via the renal artery. Comparison of the MRI response before and after injection of labeled cells into the left kidney is provided in Figure 3. One can see that the T_2-weighted image indicates some darkening around the outer cortex of the infused left kidney (arrow - right image) relative to the right or the left kidney prior to labeled cell injection. The presence of labeled cells in the region of interest resulted in an enhancement in the reduction in MRI signal intensity of water at the site due to the magnetic susceptibility (reduction in the T_2* relaxation time) produced by the encapsulated iron oxide particles.

Conclusions:

MPIOs have been shown to readily undergo efficient and non-toxic uptake by macrophages in cell culture and single labeled cells can be detected *in-vitro* at concentrations of 1.0×10^3cells/μl using MRI. *In- vivo* imaging of labeled macrophages infused into transplanted kidneys provided detectable contrast at concentrations $\leq 2.4 \times 10^6$cells/800 μl. Immune cell labeling with larger particles should alleviate the necessity of having to incorporate very high numbers of smaller particles into individual cells to allow *in-vivo* detection and thus should provide an improved ability to detect cell accumulation at the sites of transplant rejection.