

Long Term Cellular MR Imaging Using Micron Sized Iron Oxide Particles

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Introduction

Molecular and cellular imaging have made a tremendous impact in the last few years for monitoring cell migration and homing. In most cases, cells are labeled with nanometer sized dextran coated iron oxide particles *in vitro*, and administered into a host animal. There are however, two disadvantages to using dextran coated, nanometer sized particles for cell labeling. The first is that millions of particles are necessary to achieve detection. This requires highly efficient labeling schemes, and cell division can dilute the label beyond detectability. The second is that the dextran coated particles are biodegradable and after several weeks, the particle is broken down and the iron is recycled by the cell (1). Both of these disadvantages can severely hamper any study where long term engraftment and stability is being examined or very slow migration is anticipated. To alleviate these problems, cells can be with polymer encapsulated, micron sized iron oxide particles (MPIO's) (2). These particles can be detected as single particles in tissue, which avoids problems of detection due to cell division (3). Furthermore, these particles are indestructible under physiological conditions and can be detected for many months. We demonstrate these facets using two different rodent models. The first is hepatocyte transplantation therapy, where the goal is to achieve long term engraftment of donor hepatocytes. The second is neural precursor migration along the rostral migratory stream (RMS) to the olfactory bulbs (OB's), a process which can take weeks.

Materials and Methods

Mouse hepatocytes were isolated using standard methods. Hepatocytes were labeled with 0.96 micron diameter MPIO's by simple overnight incubation of confluent cell culture dishes with 10^9 particles. These MPIO's are polystyrene/divinylbenzene polymer coated iron oxide cores with a fluorescein derivative impregnated into the shell. The measured relaxivity of MPIO's at 4.7T was $356 \text{ mM}^{-1}\text{s}^{-1}$, while MION was $240 \text{ mM}^{-1}\text{s}^{-1}$ (2). Free particles were removed by rigorous washing followed by Ficoll-Paque density centrifugation. Host mice were injected in the spleen with one of the following: unlabeled cells, labeled cells or without injection at all. Hepatocytes migrate spontaneously to the liver as single cells over the course of the next few days (4). At one month and one year periods, mice were perfused, fixed with 1 mM Gd-DTPA doped 4% formaldehyde, and the livers were removed and placed in saline. 3D gradient echo MRI was performed at 4.7 or 7 Tesla (TE = 10 ms, TR = 100 ms) at 100 microns isotropic resolution. Livers were then sectioned, and also scanned intact with two-photon fluorescence microscopy.

Neural stem cells were labeled by direct stereotactic injection of 1-50 μl of a 1:1 mixture of 1.63 micron and 0.96 micron streptavidin coated particles (10^{7-8} particles) into various regions of the sub-ventricular zone. 3D gradient echo images of live, anesthetized rats were acquired at time points over the course of six weeks with the following parameters: TR = 70 ms, TE = 8 ms, 100 micron isotropic across 2.56 cm^3 . Imaging was done at 11.7 Tesla using a volume transmit and 3.5 cm receive only surface coil. Rats were then perfused, fixed with 1 mM Gd-DTPA doped 4% formaldehyde, and the brains with intact olfactory bulbs were removed. Brains were then placed in saline and imaged overnight at 50 microns isotropic resolution. Following imaging, brains were sectioned for histology, and also imaged intact using two-photon fluorescence microscopy.

Results

Figure 1A is an optical image of a hepatocyte labeled with MPIO's. More than 100 particles can be seen in the cytoplasmic space, and as each particle contains ~ 0.1 pg of iron, cells were labeled with >10 pg iron. MRI evaluation one month post hepatocyte transplantation showed scattered dark, punctuated contrast in the livers of those mice who received labeled cells (Fig. 1B). Livers of mice who received unlabeled cells (Fig. 1C) and livers from uninjected mice showed no susceptibility induced dark contrast. This was true for the one year post transplants as well, with similar counts of dark contrast regions. All mice (n=5) who underwent labeled cell injection displayed dark contrast in the livers; all unlabeled cell injection and uninjected mice were negative for contrast (n=5, n=5). Two-photon fluorescence microscopy confirmed the presence of particles in labeled cells in mice livers.

Figure 2 is a sum of five slices from a 3D *in vivo* data set, showing the frontal part of a rat brain 5 weeks post injection of 50 μl particles. Dark contrast extends from the rostral tip of the lateral ventricle, dives rostral and inferior towards the base of the brain, and curves upwards into the OB's, where the contrast spreads out and becomes punctuated (arrows). This is precisely the RMS as indicated by numerous histological methods (5). Large susceptibility effects from the ventricles is due to the large number of particles injected. The degree of labeling of this migratory pathway is dependent on injection site as well as quantity of particles injected. Increased contrast is observed with scans longer post injection as well. High resolution *ex vivo* MRI depicts the contrast diffusing into the OB's very clearly. Two-photon fluorescence microscopy confirmed the presence of particles in the interior of the OB's.

Discussion

Here we demonstrate that liver cells in culture endocytose large quantities of MPIO's and when transplanted into a host, carry the particles for at least one year. Inter-splenic hepatocyte transplantation therapy delivers hepatocytes to the liver as single cells where they migrate along ducts, so each of the dark spots in the liver images corresponds to a single cell. The key finding here is that the particles remain intact and elicit contrast over the course of the year, where dextran coated particles would have been recycled long before this. The size of individual contrast regions ranges from 100 to 300 microns. This size range is the same for the livers imaged one month and one year post transplantation. Equivalent numbers of contrast areas were observed at the two imaged time points as well.

The second key finding is that neural stem cells can endocytose particles at their origin, carry them along migratory pathways, and maintain contrast weeks later. This is the first demonstrated case of *in vivo* stem cell labeling. As only single micron size particles are required for detection, poor labeling efficiency can be tolerated and *in vivo* labeling is possible. Millions of dextran coated nanoparticles are necessary for detection, making this type of cell labeling difficult. It has been shown that neural stem cells reside in a unique microenvironment in the sub-ventricular zone. *In vivo* labeling of stem cells will be potentially important to preserve this microenvironment, making neural stem cell tracking less invasive.

References: 1) Weissleder, R, et al, *Am J Roentgenol*, **152**, 167-73, (1989), 2) Hinds, KA, et al, *Blood*, **102**:3, 867-72, (2003); 3) Shapiro, EM, et al, *Proc ISMRM 2003*, #229; 4) Gupta, S, *Semin Cell Dev Bio*, **13**, 439-446, (2002); 5) Suzuki, SO & Goldman, JE, *J Neurosci*, **23**:10, 4240-4250, (2003)

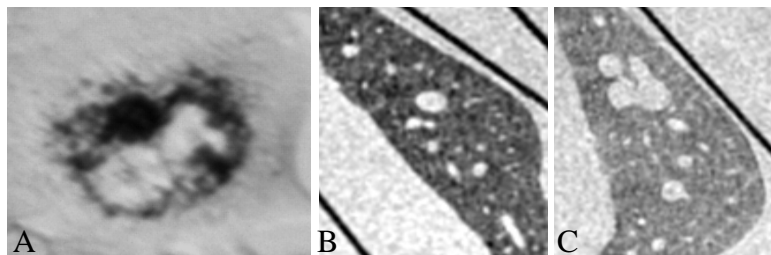


Figure 1: A) Hepatocyte labeled with >100 0.96 micron MPIO's, B) liver from mouse injected with labeled cells, C) liver from mouse injected with unlabeled cells.

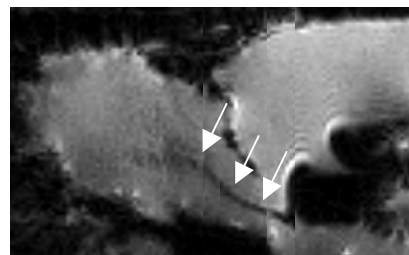


Figure 2: Sum of five slices from a 3D *in vivo* data set. Note the contrast extending from the lateral ventricle to the olfactory bulb.