IN-VIVO IMAGING OF TRANSPLANTED HUMAN HEPATIC STEM CELLS: NEGATIVE CONTRAST LABELING AND 7T MICRO-MRI TRACKING

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1. INTRODUCTION

Stem cell therapies have great potential as alternative options to whole organ transplantations in treating dysfunction or failure, and alleviating the chronic shortage of donor availability of organs such as the liver. In these therapies, adherent stem cells are transplanted to replace and repopulate diseased tissues, hence allowing the failing organs to regain their functional effectiveness. Given that clinical trials are ongoing or are about to occur with many different categories of stem cells, there is a need for *in vivo* stem cell imaging to monitor cell motility after inoculation, and to follow the location and expansion of the stem cells thereafter. Specifically, some topics being addressed comprise cell transplantation procedures, cell migration dynamics, and possibilities for creating tissue-cell-vacuums to permit engraftment and expansion of the transplanted cells. In this way, the efficacy of stem cell therapy may be evaluated and realized.

The goals of the current study are to develop effective procedures for labeling stem cells with contrast agents for magnetic resonance imaging (MRI), and to investigate the MRI detectability of labeled cells. The model systems are human hepatic stem cells that are isolated by fractionation or immunoselection technologies from human fetal livers. In brief, there are two subpopulations of human hepatic stem cells, both of which are positive for Epithelial Cell Adhesion molecule (EpCAM) as well as for other antigens (Moss, Wauthier et al. 2004). The uptake, retention and physiological effects of magnetic labels are examined with *in vitro* experiments. Subsequently, the human hepatic stem cells are transplanted into immunocompromised SCID/nod mice to monitor *in vivo* MRI cell signals. To facilitate time-lapse investigations, several animals are inoculated simultaneously but independently imaged in order to quantify signal intensities, durations, and spatial distributions of cells within resident mice livers.

2. MATERIALS AND METHODS

Two types of magnetic contrast agents were utilized in the current study, a dendrimer-coated superparamagnetic iron oxide nanoparticle (Bulte, Douglas et al. 2001), and a novel conjugate of magnetic cell sorting microbeads (Miltenyi, Auburn, CA) and HEA-125, which is an EpCAM anti-human epithelial antibody. The "magnetodendrimers" have been shown to be readily endocytosed by cells, while the microbead-antibody conjugate is expected to be a cell surface-tagging agent. Both contrast agents have particle diameters ranging from 50-100nm, which are well within virus size magnitudes of <200nm. Because these contrast agents are superparamagnetic labels, they were expected to dramatically enhance the T2* relaxation of the cells.

For the *in vitro* experiments, cells were incubated in HK medium (Kubota and Reid 2000) containing a 1.25mM concentration of magnetodendrimer for 24 hours or a 400µl/5E7cell concentration of the microbead-antibody conjugate for 30 minutes, washed to remove excess contrast agent, then embedded in 2% agarose gel. Cells similarly prepared, but without the magnetic labeling treatment, were used as control. For the *in vivo* cell tracking studies, three groups of immunocompromised SCID/nod mice were used: (a) a group where animals were inoculated with labeled stem cells, (b) a control group in which animals were inoculated with human hepatic stem cells that are not labeled, and (c) a sham group consisting of animals not exposed to stem cell inoculations but underwent all related surgical technique. Prior to MR imaging, the animals were sacrificed, exsanguination, and perfusion fixed using 4% paraformaldehyde.

High-resolution MR imaging experiments were conducted using a 7.1 T instrument (Center for In Vivo Microscopy, Duke University Medical Center, Durham, NC). For the *in vitro* studies, the cell-containing petri dishes were placed on 3.0 cm-diameter surface RF coil, and standard gradient echo images (4.0 cm FOV, 1.0 cm thick slice, 256 x 256 matrix size, TR = 250 ms, TE = 15 ms, 2 averages) were obtained. For the fixed whole mouse experiments, a 4.0 cm-diameter birdcage RF coil was used, and interleaved multislice gradient echo images (1.0 mm slice thickness, 4.0 cm FOV, TR = 250 ms, TE = 15 ms, 4 averages) spanning the length of the animal torso were acquired.

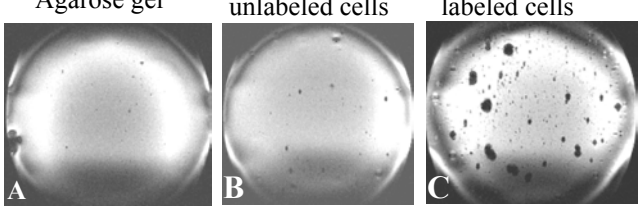

Figure 1: A) Agarose gel only. B) Agarose gel with unlabeled cells. C) Agarose gel with labeled cells.

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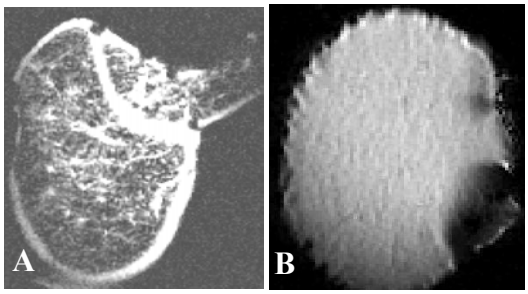
3. RESULTS AND DISCUSSION

Figure 1 shows representative results of the *in vitro* experiments. In Figure 1A, the white background represents a homogeneously dispersed gel with few void artifacts likely due to air bubbles formed during the gelling process. Next in Figure 1B, unlabeled stem cells within the gel appear similar to the gel-only sample, and are not detectable. In contrast, magnetodendrimer-labeled stem cell aggregates dispersed within the agarose gel are clearly detectable as signal voids in Figure 1C. Cells imaged 14 days after exposure to superparamagnetic contrast agents were equally detectable, suggesting that the cells retained the contrast agent, and that the contrast agent had no apparent adverse physiological effect on the cells over the time period. Similar results were obtained for cells labeled with microbead-antibody conjugates. These findings demonstrate that human hepatic stem cells can be "tagged" and detected with equal effectiveness using either magnetodendrimers or the novel microbead-antibody conjugates. However, the latter is expected to be useful in 'not only' studies involving pre-labeled cells, but also in post-transplantation monitoring that exploits the specificity provided by the EpCAM antibody.

Because labeled cells are detected primarily by their enhanced T2* relaxation (i.e., by hypointense signal in gradient-echo MRI), a particular concern for imaging fixed animals especially in the liver is the oxygenation-dependent contrast of deoxygenated blood (Ogowa, Lee et al. 1990). As shown in Figure 2A, a mouse liver lobe MR



Delayed Perfusion | Immediate Perfusion

Figure 2: Mouse liver Lobes with different perfusion applications.

imaged after delayed perfusion exhibits marked heterogeneous backgrounds in response to deoxygenated blood. Alternatively, as shown in Figure 2B, a liver lobe prepared by perfusion fixation (exsanguinated in the process) is able to achieve the appropriate background conducive for detecting magnetically labeled cells.

Figure 3A is a representative image of a mouse liver transplanted with magnetodendrimer-labeled cells. Here, cross-sections of two liver lobes are displayed with a highlighted left lobe region. Within this highlight region, multiple void signals representative of tagged human stem cells previously dispersed inside the liver are clearly visible. The pixels of labeled cells were identified by intensity thresholding using metamorph image processing software (Universal Imaging Corp, Downingtown, Pa.), as shown in Figure 3B. The physical dimension of each image pixel corresponds to an aggregate of 384 packed cells, which have diameters ranging from 7 – 10 μm. Since superparamagnetic contrast agents have effective radius larger than their physical size, the cell count is an upper-bound estimate for the minimum cell aggregate size detectable by MRI at 7.1 T.

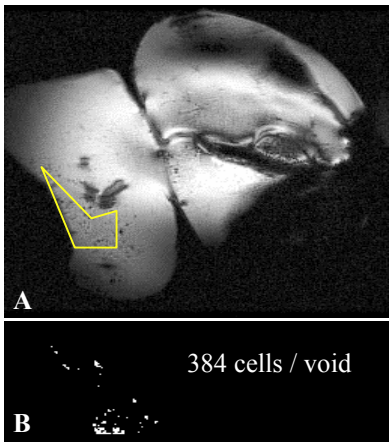


Figure 3: A) Immediately perfused mouse liver lobes with tagged human stem cells. B) Threshold images of tagged cells for quantification.

4. SUMMARY

In this work, purified human hepatic stem cells, EpCAM+ cells, were labeled to induce magnetic resonance imaging contrast by either magnetodendrimers or a novel microbead-antibody conjugate. The *in vitro* controls confirmed that imaged void spaces represent labeled cells visualized on homogeneous light backgrounds. By applying these techniques to *in vivo* studies, the labeled human hepatic stem cells were tracked throughout liver lobules enabling one to quantify cell distribution, density, and tissue mass. These variables are essential to be able to analyze the efficacy of stem cell therapies.

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