SPECIFICITY CELL LABELING for HUMAN HEPATIC STEM CELLS: 7.1T MICRO-MRI TRACKING

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Introduction: The advent of adherent stem cell therapies (currently in various stages of clinical trials) has necessitated noninvasive procedures to monitor the distribution and growth of the transplanted cells. Although a wide variety of labeling contrast agents (both iron oxide nanoparticle [1, 2] and gadolinium-based [3]) is available, the utility of MRI in monitoring cell motility is ultimately determined by the specificity and efficacy of the agents. Antigenic profiles of human hepatic stem cells, including epithelial cell adhesion molecule (EpCAM), have recently been characterized [4]. In this study, we established the protocols and demonstrate the effectiveness of EpCAM-linked magnetic nanoparticles for detecting hepatic stem cells in both *in vitro* and small animal model experiments.

Materials and Methods: A novel conjugate of EpCAM anti-human epithelial antibody, HEA-125, and magnetic cell sorting microbeads was obtained from a commercial source (Miltenyi, Auburn, CA). The NMR properties of magnetic microbeads are expected to be similar to those of superparamagnetic iron oxide (SPIO) nanoparticles. Human hepatic stem cells were isolated from fetal livers as described previously [4]. For the *in vitro* experiments, maximum concentration of contrast agent and exposure time for maintaining cell viability were determined. Specimens were prepared using cells embedded in 1% agarose on 35 mm-diameter Petri dishes. Projection 2D gradient echo images (4.0 cm FOV, 15 ms TE, 250 ms TR, 30° flip angle) were obtained using a 7.1 T MR microscopy instrument for 52specimens with agarose-only (no cells), unlabeled and labeled cells.

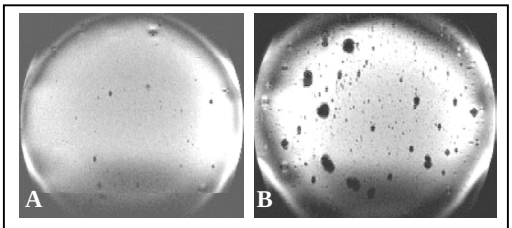
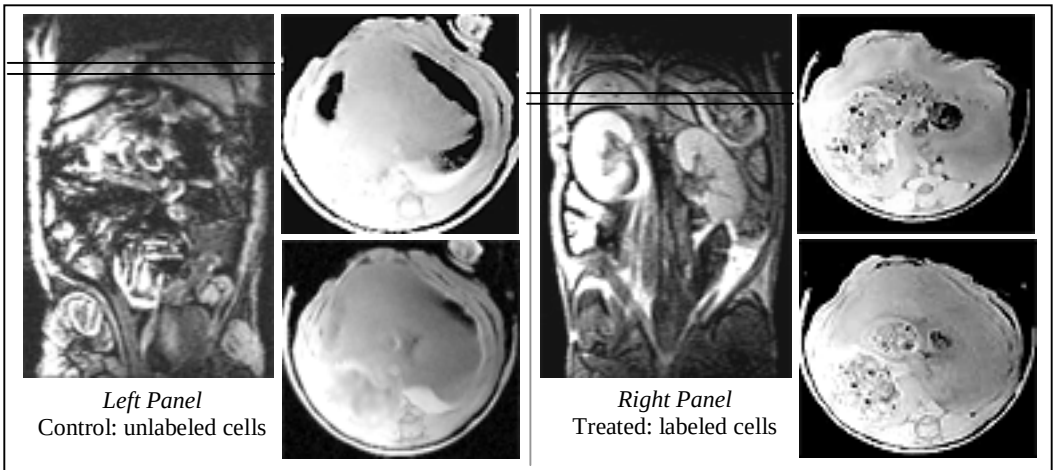


Figure 1: *In vitro* MRI of embedded liver stem cells in agarose gels. A.) unlabeled cells. B.) labeled cells.

Three groups of animals (male SCID/nod mice, minimum n = 8 each), sham (mock surgery), control (transplantation with unlabeled cells) and treated, were subsequently prepared. Transplantation consisted of intrasplenic injection of 1.5E6 cells suspended in 70µl cell culture medium. After a minimum of 3 days recovery period, prior to imaging, the animals were sacrificed, exsanguinated by saline flush, and perfusion fixed with 1:25 solution of Magnevist in formalin. Spin echo 3D images (4.0 cm in-plane FOV, 0.5 mm slice thickness, 100 ms TR, 15 ms TE, 4 averages) spanning the length of the animal torso were acquired.

Results and Discussion: For microbead cell labeling, the maximum density concentration was determined to be 100µl HEA microbeads per 5E7 cells with an exposure time of 40 minutes, for sustain long-term cell viability. As shown in Fig. 1, and compared to the control plate with unlabeled cells, cells labeled with the EpCAM-magnetic bead conjugate are clearly detectable by their enhanced relaxation. This appearance is consistent with cells labeled with SPIO-based contrast agents. Figure 2 shows coronal (with cross-section locations indicated) and axial slices images of representative animals from the control (left) and treated (right) groups. Compared to the control animal, the liver of the treated animal is studded with hypointense foci. Inferring from results seen in Fig. 1 and histology performed on separate animals, these hypointense foci correspond to aggregates of labeled human liver cells.



Left Panel
Control: unlabeled cells

Right Panel
Treated: labeled cells

Figure 2: MRI of total body perfusion and using SCID/nod mouse. Left Panel.) Control mouse containing unlabeled stem cells. Right Panel.) Treated mouse with labeled stem cells.

Conclusion: We have established cell labeling protocols and shown that cell-specific EpCAM conjugated with magnetic beads can be used to detect human hepatic stem cells in vitro and in animal models. These findings pave the way for in vivo monitoring of post-transplantation cell distribution and integration in stem cell therapy.

References:

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