In vivo Tracking of Endothelial Progenitor Cells in a Mouse Model of Choroidal Neovascularization

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Introduction - Age-related macular degeneration (ARMD) represents the leading cause of vision loss in people over the age of 50 in developed countries. The "wet" form of ARMD is characterized by choroidal neovascularization (CNV). One of the main functions of circulating endothelial progenitor cells (EPCs) is to repair the damaged vasculature. In this study we implemented an innovative method to track *in vivo* migration and engraftment of EPCs to the area of injury in a mouse model of CNV by magnetic resonance (MR) imaging techniques. Monocrystalline iron oxide nanoparticles (MION) have been shown to magnetically label cells to visualize them in vivo using MR imaging.

Materials and Methods: ***MION synthesis*:** MION was prepared using previously published protocols (2). ***Mice:*** CNV was induced in C57BL/6J by laser rupture of Bruch's membrane. ***Cell Migration Assay:*** 5,000 $CD34^+$ cells (per condition) were incubated with 1:2000, 1:1000, and 1:500 dilution of MION overnight in 5% CO_2 at 37°C. The cells were stained with Calcein-AM and loaded onto the top chamber of a disposable Boyden migration chamber and challenged to migrate towards 10% FBS or PBS as negative control. ***Cell Viability and Apoptosis:*** The effect of MION labeling on cell viability and apoptosis was tested using Annexin V-FITC apoptosis detection kit. ***In vitro imaging*:** To test the ability to detect single labeled cells, $CD34^+$ cells were loaded with MION and dispersed at very low cell density in 2% agar and loaded into 5mm NMR tubes. Samples were imaged using T2* weighted gradient echo imaging techniques (FOV 19x19 mm; matrix 512x400; thickness 180 µm; TE 15 ms). ***Ex vivo imaging*:** $CD34^+$ cells were loaded with MION prior to intravitreal injection. Mice were sacrificed 24 h later, eyes enucleated, processed, and placed in a 5mm custom built coil (Dr. Samuel Grant, UF). High-resolution 3D gradient echo imaging (TR 0.5 sec; TE 7.5 ms; matrix 200x180x80; FOV 3.8x3.8x3.8 mm; NA 6) was used for cell tracking.

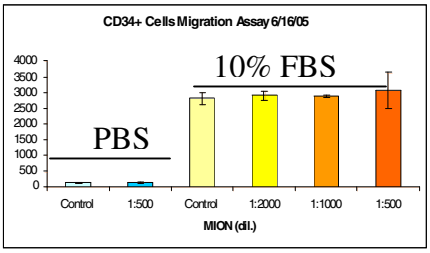

Figure 1. **Cell Migration Assay.** Increasing MION did not affect cell migration towards 10% FBS

Results: ***Cell Migration Assay:*** Increasing the MION concentration did not have any effect on cell migration compared to sham-treated cells (Fig.1). ***Cell Viability and Apoptosis:*** MION labeling did not induce apoptosis of EPCs compared to controls (data not shown). ***In vitro imaging*:** MION-labeled cells were detectable on a 17.6 Tesla magnet at all three incubation conditions (dilutions) of MION. ***Ex vivo imaging:*** The extraordinary resolution (in-plane of 20 μm^2) clearly showed at least six of the retinal layers in the excised eyes (Fig.3 A), and MION-labeled EPCs were detectable in the retina twenty four hours post-intravitreal injection (Fig.3 B). Histology is currently being conducted to confirm that the migration is to the site of retinal injury.

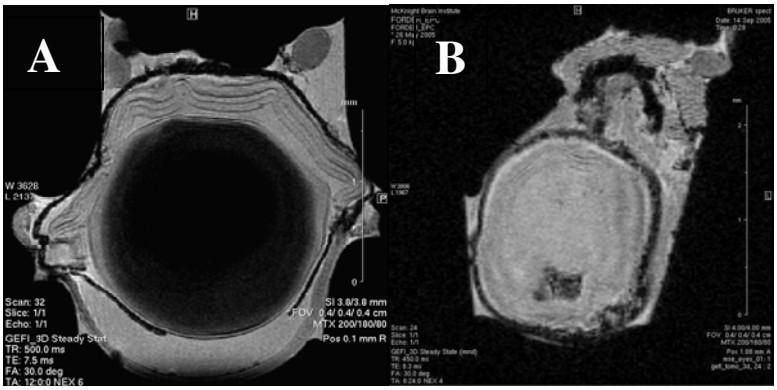

Figure 3A,B. High resolution gradient echo images of excised murine eye. Approximate in-plane resolution is 20 μm^2 and thickness is 40 µm. Panel A shows control conditions, while panel B shows decreased image intensity associated with MION labeled cells.

Conclusions: In this study we implemented an innovative method to track *in vivo* migration and engraftment of EPCs to the area of injury in a mouse model of choroidal neovascularization by magnetic resonance imaging techniques. Labeling EPCs with MION does not affect their physiology, while it provides an excellent MR imaging contrast. This technique, offering the advantage of non-invasively monitoring of *in vivo* cell migration, will open a new avenue in the treatment of ocular dysfunction.

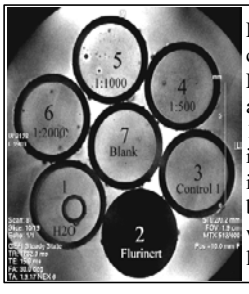

Figure 2. Representative image of cell detection agarose gel. Following incubation with MION at dilutions of 1:500, 1:1000, or 1:2000, cells were diluted to yield individual cells in suspension and imaged. Clockwise from the bottom are tubes of fluorinert[a], water standard, increasing MION loading of EPCs, and control.

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