In Vivo Magnetic Resonance Imaging of Embryonic Stem Cells in Mouse Myocardial Infarction Model

N. Himes[1], J. Min[2], R. Lee[2,3], C. Brown[2,3], J. Shea[2,3], M. Takahashi[4], J. Morgan[2], P. Oettgen[2,3], D. Burstein[1,4]

[1]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA, United States, [2]Cardiology, Beth Israel Deaconness Medical Center, Boston, MA, United States, [3]New England Baptist Bone and Joint Institute, Boston, MA, United States, [4]Radiology, Beth Israel Deaconness Medical Center, Boston, MA, United States

Introduction:

The potential of administering stem cells to promote angiogenesis and myocardial tissue regeneration after infarction and the ability to track adult stem cells in large animal models has recently been demonstrated[1]. However there are advantages in using embryonic stem cells for research and eventual clinical application; depending on the culture conditions, differentiation along specific lineages can be promoted so that certain subsets of cells could be isolated depending on the therapeutic application. Also, unlike primary adult stem cells, embryonic stem cells could be engineered to express factors such as angiogenic growth factors or other proteins that might promote myocardial tissue regeneration. Furthermore, it is desirable to track cells in mouse hearts which allows for high throughput and the study of transgenic models of cardiac disease. The aims of this study were **(i)** to determine if mouse embryonic stem (ES) cells could be loaded with superparamagnetic iron oxide particles (SPIO) and if the SPIO particles remain within the cells in culture and in the myocardium and **(ii)** if these cells could be detected by MRI in a mouse model of myocardial infarction for both short and longer time points.

Methods:

(i) Determine if mouse ES cells could be loaded with SPIO particles and if the iron oxide particles remained in the cells:

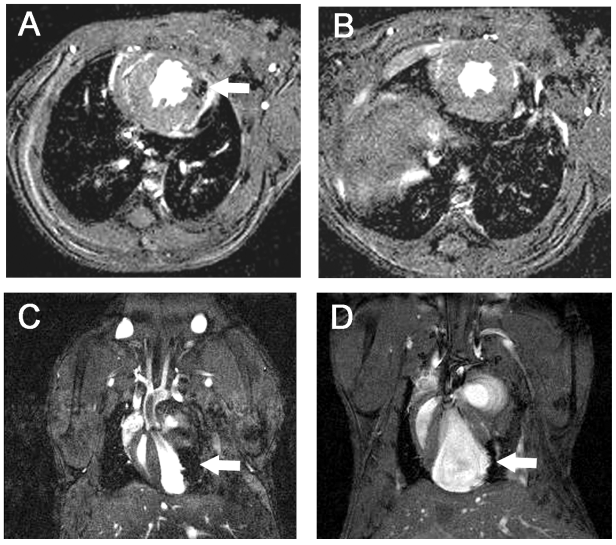
A transfection solution containing 6 μl/ml PLUS Reagent and one of the following three different concentrations of Feridex (Advanced Magnetics): 11.2 μg Fe/ml, 22.4 μg Fe/ml, and 44.8 μg Fe/ml in serum free medium was prepared and 4 μl/ml lipofectamine was added. Mouse ES cells were incubated in 1 ml of transfection solution and lipofectamine at 37° for 4 hours. Feridex labeled ES cells were harvested, counted, and diluted to a final concentration of $1x10^7$ cells/ml in cell culture medium. Prussian blue staining was performed to evaluate iron oxide uptake in cells.

A subset of mouse ES cells incubated with the highest Feridex concentration of 44.8 μg Fe/ml were also labeled using the nuclear stain 4', 6-diamidino-2-phenylindole (DAPI) at a concentration of 5 μg/ml in serum free knockout DMEM at room temperature for 15 minutes. Cells were counted and a solution diluted to a final concentration of $1x10^7$ cells/ml in cell culture medium was prepared. Immediately after inducing myocardial infarction (MI) in age-matched mice (n=9) through ligation of the left anterior descending artery, injections of 10 μl each of the mouse ES cell solution were made into the middle of the infarcted region and the upper and lower ischemic borders. Mice were sacrificed and hearts were excised at 24 hours post-injection of the Feridex/DAPI labeled mouse ES cells. Excised hearts were fixed and cut into 5μm serial sections and DAPI visualization and Prussian blue staining was performed on each section.

(ii) Determine if SPIO labeled mouse ES cells could be detected at both short and long time points using MRI:

For *in vivo* MRI studies, age-matched infarcted mice (n=8, distinct from mice described previously) with Feridex labeled mouse ES cells injected into the myocardium were prepared using the same infarction, magnetic labeling (using 44.8 μg/ml Feridex in serum free medium), and injection techniques described above. Age-matched control mice (n=2) were prepared by injecting 10 μl of only cell-free medium into the same three locations of the myocardium as that used for the mouse ES cell injected mice. MRI studies were performed on a 4.7-T horizontal-bore MR scanner (Bruker Instruments, Billerica, MA) using an ECG and respiratory-triggered gradient echo sequence with the following parameters: TE, 3.3 ms; TR, 1RR interval; maximal in-plane resolution, 98 μm^2. Total acquisition time per slice was approximately 1m42s with some variability due to triggering. The eight Feridex labeled mouse ES cell injected mice were imaged as follows: two mice were imaged at 1 day post-injection and then sacrificed, two mice were imaged at 1 and 2 days post-injection and then sacrificed, two mice were imaged at 1, 7, and 15 days post-injection and then sacrificed, and two mice were imaged at 1 and 36 days post-injection and then sacrificed. The two control mice were imaged at 1day post-injection and then sacrificed.

Results:



(i) Iron oxide staining demonstrated that mouse ES cells took up the Feridex particles and that increasing concentration of Feridex particles in the culture medium resulted in increasing concentrations of intracellular Feridex. Colocalization of staining on Prussian blue and DAPI of infarcted hearts 24 hours post-injection demonstrated that the Feridex was localized to regions of the embryonic stem cells, and thus the cells maintained the particles *in vivo*.

(ii) The MR signal intensity throughout the heart was not impacted in the control mice by the cell-free medium. However, a large susceptibility effect was observed in all eight mice injected with Feridex labeled mouse embryonic stem cells (Fig. 1). This susceptibility effect remained relatively consistent in size for the different time points studied with a slight narrowing and elongation of the effect at the longer time points perhaps as a result of ventricular remodeling.

Figure 1: Comparing Feridex labeled mouse embryonic stem cells to cell free medium injected mice (A and B) and longevity of the susceptibility effect of Feridex labeling of mouse embryonic stem cells (C and D): Short axis T1-weighted *in vivo* MR images of a mouse 7 days post-infarct and injection of Feridex labeled mouse embryonic stem cells (A) and a control mouse 1 day post-infarction and injection of cell-free medium (B). Note the distinct susceptibility effect (arrow) observed in the mouse with Feridex labeled cells that is not observed in the control mouse. Long axis T1-weighted *in vivo* MR images of the same mouse at 1 day post-infarct and injection of Feridex labeled mouse embryonic stem cells (C) and again at 36 days (D). Note that the susceptibility effect from the Feridex labeled cells (arrows) is still distinct at the 36 day time point.

Discussion:

Our results demonstrate that loading embryonic stem cells with SPIO particles and visualizing the presence of these cells long term *in vivo* in the mouse is feasible. At the level of loading and volumes of mouse embryonic stem cells injected in our study in relation to the small size of the mouse heart, we were not able to clearly distinguish the distinct injection sites, which might be better distinguished if a smaller volume of injection or a smaller concentration of Feridex is used for loading of the cells.

References:

1. Kraitchman DL, et al., Circulation 2003; 107:2290-3