In vivo MR evaluation of the timing of mouse embryonic stem cell transplantation at 4.7 T

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Introduction: While recent medical advances have improved the treatment of congestive heart failure (CHF), the disease continues to be a major public health problem. Stem cell transplantation may offer therapeutic potential to salvage the injured myocardium. We have previously reported *in vivo* longitudinal MRI evaluation of improved cardiac function in acute myocardial infarction (AMI) mouse model following direct transplantation of mouse ESC (mESC) [1]. Here, we investigated the timing of direct mESC transplantation after myocardial infarction on mouse cardiac function using 4.7 T.

Methods: The mESC line, TL-1, (derived from 129Sv/J mice) was cultured in DMEM supplemented with 15% fetal bovine serum, L-glutamate, penicillin, streptomycin, 0.1mM B-mercaptoethanol (Sigma, St. Louis, MO) and 10^3 μ/ml leukemia inhibitory factor (Chemicon International, Temecula, CA). Cells were labeled using 25-ug of Feridex (Berlex Laboratories, Wayne, NJ) and 375-ng of PLL (Sigma, St. Louis, MO) for 1 cc of mESC medium and incubated with mESC for 12 hours [2].

The mice (129Sv/J) weighing 18-23 grams were anesthetized, endotracheally intubated and ventilated on Harvard rodent ventilators. A left thoracotomy was performed followed by ligation of the mid-left anterior descending artery. 250,000 syngeneic mESC were then injected into the infracted myocardium for 0-hour transplantation group only. A thoracostomy tube was placed, incision closed, mice were then extubated and allowed to recover. For the 96-hour group, a repeat thoracotomy was performed to inject 250,000 labeled mESC. Seven 129Sv/J mice were imaged using a Unity Inova console (Varian, Inc., Palo Alto, CA) controlling a 4.7T, 15cm horizontal bore magnet (Oxford Instruments, Ltd., Oxford, UK) with GE Techron Gradients (12G/cm) and a volume coil with an inner diameter of 3.5cm (Varian, Inc., Palo Alto, CA). The numbers of mice in 2 transplantation time points including one non-treated AMI mouse (control) per group are the following: 0-hour (4) and 96-hour (3). The mice were anesthetized using isofluorane. The ECG gating was optimized using 2 subcutaneous precordial leads with respiratory motion and body temperature monitoring (SA Instruments, Inc., Stony Brook, NY). Viability was assessed using ECG-triggered delayed-enhanced imaging acquired 30-50 minutes following intraperitoneal injection of 0.3mmol/l/kg Gd-DPTA (TE 2.8-ms, TR 30-ms, FA 90°, FOV 3.0 cm^2, matrix 128x128, slice thickness 1.0-mm, and 8 NEX) [4]. LV function was evaluated using ECG-triggered cine sequence (TE 2.8-ms, TR 160-ms, FA 60°, FOV 3.0cm^2, matrix 128×128, slice gap 0-mm, slice thickness 1.0-mm, 8 NEX, and 12 cardiac phases). Imaging plane was localized using scout images in a coronal plane followed by double-oblique acquisition.

Data were analyzed using MR Vision software (Winchester, MA). LV ejection fraction (LVEF) was calculated by tracing the endocardial and epicardial borders in end-systolic and -diastolic phases. Infarct volume was measured by adding the traced area of delayed-enhancement in each slice.

Results: This study demonstrated reliable *in vivo* longitudinal assessment of viability and cardiac function at 4.7 T. The MR evaluation at 2 weeks indicated significant restoration of LVEF in the group undergoing mESC transplantation at 0-hour and not at 96-hour post-AMI. The mESC treated vs. non-treated groups demonstrated mean LVEF of 56% vs. 34% ($p<0.05$) and 44% vs. 38% ($p>0.05$) in 0- and 96-hour, respectively. In addition, a non-significant increase in LVEF of 56% vs. 44% ($p>0.05$) was observed in the earlier 0-hr transplantation when compared to 96-hr group. Delayed-enhanced images demonstrated negative correlation $r= -0.7$ of the infarct size with mESC-treatment. The end-diastolic and end-systolic MR images of the mESC-treated and non-treated groups are shown in Figure 1. Cell labeling (white arrow) and the corresponding infarct territory at 2 week are shown in Figure 2.

Conclusions: Longitudinal *in vivo* MR assessment of viability and LV function at 4.7 T in mouse AMI model has been demonstrated. Transplantation therapy of mESC appears to be more at an earlier time point following AMI. Evaluation of larger sample size and more time points for mESC transplantation is currently underway.

References: [1] Arai, T et al. *J Am Coll Cardiol* 2004 (accepted) [2] Frank, JA et al. *Radiology* 2003;228:480-487

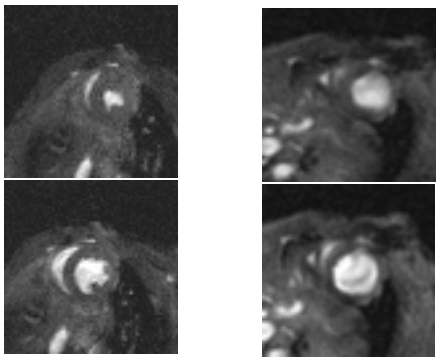

Fig. 1: End-systolic and –diastolic images of mESC-treated (left) and non-treated mouse myocardium (right).

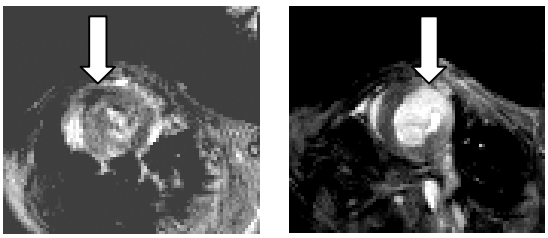

Fig. 2: Labeled mESC is detected in the area of infarct (left). Delayed-enhancement is seen in the corresponding territory (right).