In vivo MR evaluation of the restorative effects of 3 mESC types in mouse model of acute myocardial infarction

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Introduction: We have previously reported that *in vivo* MRI demonstrated improved cardiac function in acute myocardial infarction (AMI) mouse model following direct transplantation of undifferentiated mouse embryonic cells (mESC) [1]. However, optimal cell type of mESC for myocardial restoration is not known. In this study, *in vivo* MRI was performed at 4.7 T to evaluate the restorative effects of 3 mESC types in acute AMI mouse model: 1) undifferentiated, 2) differentiated, and 3) Bcl2 (anti-apoptotic) transfected mESC.

Methods: Undifferentiated D3 mESC derived from 129/Sv+c/+p mice were cultured in supplemented DMEM solution with 10^6 u/ml leukemia inhibitory factor (Chemicon International, Temecula, CA). Differentiated mESC-derived cells were generated by aggregating undifferentiated mESC in an embryoid body (EB) using the hanging drop method. Spherical EB flooded with 0.5 ml of ESC differentiation media contained small beating patches of cells at day 9. These beating cells were dispersed and plated at a density of $5x10^5$ cells/well. Bcl2-mESC clones were established by co-transfecting undifferentiated D3 mESC cells with the human Bcl2-IRES-EGFP transgene. Cells were selected with puromycin and resistant clones were picked and checked for expression. All the cells were labeled using 25-ug of Feridex (Berlex Laboratories, Wayne, NJ) and 3μg/ml of protamine sulfate (American Pharmaceutical Partner, Schaumburg, IL) and incubated for 12 hours [2]. The mice (SCID beige) 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 mESC were then injected into the infarcted myocardium. A thoracostomy tube was placed, incision closed, and mice were extubated. Twenty-eight SCID beige mice (8 mice/cell type and 4 AMI for negative control) 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 ECG gating was optimized using 2 subcutaneous precordial leads with respiratory motion and body temperature monitoring (SA Instruments, Inc., Stony Brook, NY). 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. The data were analyzed using MR Vision software (Winchester, MA). LV ejection fraction (LVEF), end-diastolic (LVED), and end-systolic (LVES) volumes were calculated by tracing the endocardial and epicardial borders in end-systole and -diastole.

Results: This study demonstrated reliable *in vivo* longitudinal assessment of cardiac function at 4.7 T following cell transplantation. The MR evaluation at 1 and 2 weeks indicated significant restoration of LVEF in all 3 cell-treated groups in comparison to the non-treated group. The treated vs. non-treated groups demonstrated mean LVEF of 38%, 40%, and 41% vs. 26% ($p<0.05$) at week 1 and 32%, 33%, and 31% vs. 26% ($p<0.05$) at week 2 in undifferentiated, differentiated, and Bcl2 groups, respectively (Figure 1). There was no significant difference in the LVEF among the 3 treatment groups (Figure 2). In addition, efficient *in vivo* labeling of undifferentiated and differentiated mESC by Feridex was also seen (Figure 1).

Conclusions: Longitudinal *in vivo* MR assessment of cell therapy is possible. Restoration of myocardial function was seen in all 3 cell types. Simultaneous detection of labeled cells and assessment of LV function at 4.7 T in mouse AMI model enables monitoring of therapeutic progress.

[1] Arai, T et al. *J Am Coll Cardiol* 2004;43(Suppl A):532A.
[2] Frank, JA et al. *Blood* 2004 104(4):1217-23

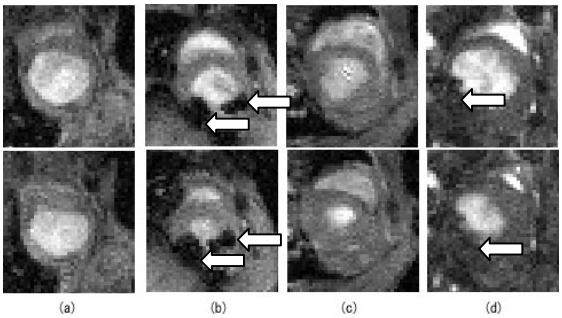

Fig. 1: End-diastolic and -systolic images of (a) non-treated AMI (negative control), (b) undifferentiated mESC, (c) differentiated mESC, and (d) Bcl2 mESC. The dephasing signal from the labeled cells are shown (white arrow).

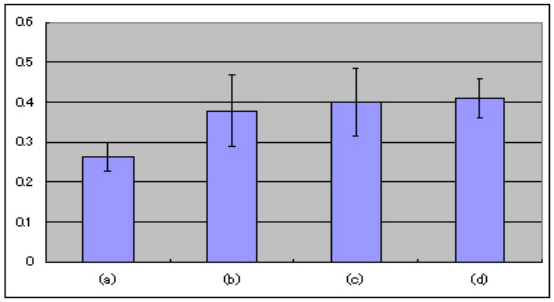

Fig. 2: Bar graph of the LVEF of 4 different groups (a) non-treated AMI (negative control), (b) undifferentiated mESC, (c) differentiated mESC, and (d) Bcl2 mESC.