In vivo MR evaluation of embryonic cardiomyoblast tissue graft in rodent acute myocardial infarction model of heterotopic heart transplantation

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Introduction: Tissue grafting of stem cell may provide an alternative therapy to restore the infarcted myocardium. A novel bioartificial tissue graft of embryonic cardiomyoblast in acute myocardial infarction (AMI) rodent model of heterotopic heart transplantation has been reported (1). In this study, the feasibility of *in vivo* MRI to evaluate the restorative effects of embryonic cardiomyoblast tissue graft in heterotopic heart was investigated.

Methods: Male Lewis rats underwent sternotomy followed by ligation of the mid-left anterior descending artery to achieve an extensive left ventricular infarction. The hearts were perfused with cardioplegic solution and harvested in a standard fashion. *Ex situ*, a piece of collagen matrix (3x3x2 mm) was implanted into the infarcted area of left ventricular (LV) wall. Following anastomosis of the pulmonary artery to the left atrial appendix, the heart was transplanted into the abdomen of syngeneic animals. An aorto-aortic anastomosis and an anastomosis of the donor heart superior vena cava to the recipient abdominal vena cava were established to create a functional heart model. Before reperfusion, a suspension of $1x10^6$ embryonic cardiomyoblasts (H9c2) and liquid collagen was injected into the reconstructed area to form an embryonic cardiomyoblast tissue graft. Twenty-one Lewis rats underwent MRI at 4-week following heterotopic transplantation. The rats were divided into 4 groups (5-6 rats/group): 1) heterotopic heart transplant (HTp); 2) HTp with LAD ligation (HTp-AMI); 3) HTp-AMI-H9c2-collagen; and 4) HTp-AMI-H9c2-collagen-VEGF. The 4.7 T MRI was performed using a Unity Inova console (Varian, Inc., Palo Alto, CA), 15cm horizontal bore magnet (Oxford Instruments, Ltd., Oxford, UK) with GE Techron Gradients (12G/cm), and a volume coil with an inner diameter of 5cm (Varian, Inc., Palo Alto, CA). The rodents 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). 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 at 4.7 T of the cardiac function in a rodent heterotopic heart transplant model. The MR evaluation indicated significant restoration of LVEF following AMI by H9c2 tissue grafts. The H9c2 tissue graft treated vs. non-treated groups demonstrated mean LVEF of 45% vs. 32% ($p<0.05$), respectively, at week 4 as shown in Figure 1. However, the mean LVEF of normal heterotopic heart transplant control group was significantly higher than those of the HTp-AMI-H9c2-collagen group (57% vs. 45 %, $p<0.05$). Additional VEGF treatment did not confer any further improvement in LVEF as shown in Figure 2.

Conclusions: Longitudinal *in vivo* MR assessment of heterotopic heart transplant is possible. Restoration of myocardial function was demonstrated by H9c2 bioartificial tissue grafting.

[1] Kutschka et al., International Society of Heart Lung Transplant, 2005 (accepted)

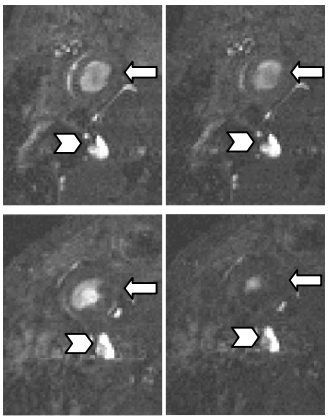

Fig. 1
(a) End-diastolic and -systolic images of HTp-AMI (white arrow) and abdominal aorta (white arrowhead)

(b) End-diastolic and -systolic images of HTp-AMI-H9c2-collagen (white arrow) and abdominal aorta (white arrowhead)

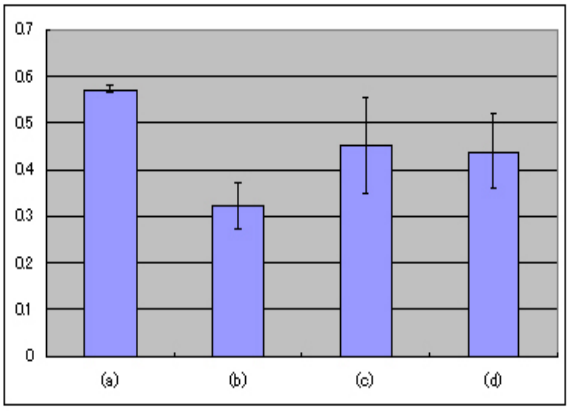

Fig. 2: Bar graph of the LVEF of 4 different groups (a) HTp, (b) HTp-AMI, (c) HTp-AMI-H9c2-collagen and (d) HTp-AMI-H9c2-collagen-VEGF