Three Dimensional Tracking of Stem Cells Grafted in the Infarcted Myocardium

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Introduction Rodent myocardial infarction (MI) models have been used frequently in preclinical evaluations of therapies that aim to prevent or reverse the post MI- remodeling process which leads to heart failure. Stem cells therapy has been proposed as a restorative treatment. Stem cells labeled with superparamagnetic iron oxide (SPIO) particles may be detected by MR imaging after engraftment into infarcted myocardium of large [1] and small animals [2]. In this study, we used in vivo and ex vivo 3D MR imaging to map the distribution of these grafted cells. Histological staining of IO was included to confirm the localization of SPIO particles.

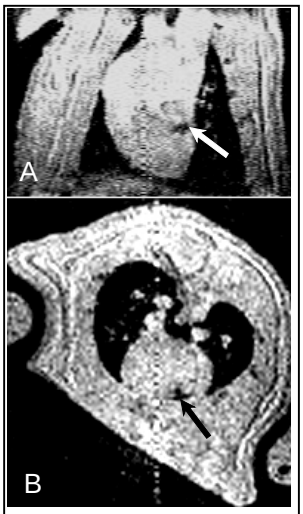

Fig.1. In vivo images of long (A) and short (B) axis view of the heart injected with 3E6 cells.

Methods Left ventricular myocardial infarction was generated in male CD-1 rats by ligation of the left anterior descending artery for 1 hour followed by reperfusion. H9c2 cells (rat embryonic cardiomyoblasts) were incubated for 36 hours in labeling media containing 50 µg/ml SPIO particles (Feridex®) in addition to 0.4 µg/ml of poly-L-Lysine. Various numbers of cells in 50-30 µl (3E6, 3E5, 3E4 and 3E3) were injected in the infarcted region when perfusion was resumed. MR images were acquired using a 60-mm volume coil interfaced to a 4.7T horizontal bore Varian INOVA spectrometer. The animal was anesthetized with 1-3% isoflurane during MRI and its ECG, respiration and core temperature were monitored (SA Instruments, Stony Brook, NY) with core temperature maintained at 37±0.2°C and heart rate at 370±40 bpm. ECG-gated 3D GRE data were acquired from a 3D slab of the rat expanding the heart left ventricle region of the chest (TR is about 0.12-0.15s dictated by heart rate, TE=0.005s, flip angle=20°, acquisition is about 1 hour FOV=7x3.5x2 cm^3 with a matrix=128 x128 x 64). The heart was then removed and perfused retrograde through the aorta with saline, fixed with formalin, and immersed in Fomblin LC08 (Solvay Solexis Inc. NJ) for ex vivo imaging. A non-slice-selective 3D gradient echo sequence was used to acquire the data (TR=0.1s, TE=0.01s, matrix= 256^3 from a FOV of 2^3 cm^3, signal average=12, acquisition time=13 hours). Histology was performed on formalin fixed heart. For Prussian blue staining, slides were incubated with Perls' reagent consisting of 2% potassium ferrocyanide (Sigma-Aldrich Co, St. Louis, MO) in 23.4% HCl for 30min in a dark room followed by counterstaining with nuclear fast red (Sigma-Aldrich).

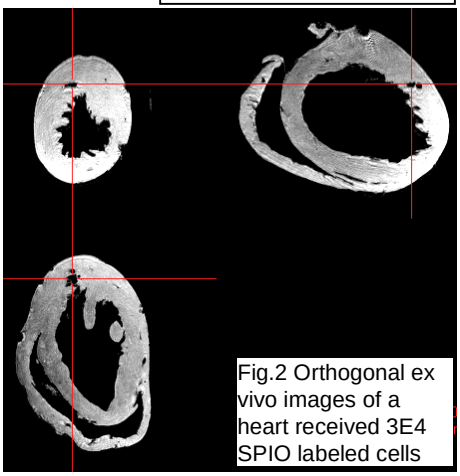
Fig.2 Orthogonal ex vivo images of a heart received 3E4 SPIO labeled cells

Results Long (Fig.1A) and short (B) axis views of the heart were extracted from an in vivo 3D data set with arrows pointing to SPIO labeled cells. Three orthogonal views of an ex vivo heart receiving 3E4 cells were shown in Figure 2. The 3D data set facilitates the localization of labeled cells by co-registering them on orthogonal images, thus minimizing the risk of false positive results. *In vivo* detection was limited by S/N and was not able to detect 3E4 or fewer cells while the 3E3 cells were readily detected by ex vivo imaging. The spatial resolution of 78 µm was achieved in ex vivo imaging and together with the lower detection limit would permit mapping the potential migration of injected cells. SPIO labeled cells were identified by Prussian blue staining and confirmed by their unique morphology (round shape and large nuclei).

Discussion One limitation with SPIO labeling (or other labeling methods) is the consequence of cell lysis and absorption of the iron label by other tissues/cells. Therefore, this method may not be suitable for detection of the survival status of the grafted cells. However, SPIO labeling using a FDA approved contrast agent and the labled cells can be visualized by high resolution MRI, and thus is readily applicable to clinical studies. The effect of iron overloading on cell growth and cardiomyocyte differentiation is being investigated.

References 1. *Circulation* **107** 2290-2293 (2003). 2. *Magn Reson Med* 52 1214-1219 (2004).

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