Regenerative Myocardial Tissue in a Murine Infarction Model

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Introduction: The advent of mouse transgenic technology has emphasized the importance of high field murine cardiac imaging. The ability to serially track a genetically altered model non-invasively has proven invaluable in understanding function and anatomy at both a tissue and whole organ level. The MRL/MpJ$^{+/+}$ mouse strain has been shown to regenerate tissue during wound healing. In particular is its regenerative response to cryogenically induced myocardial injury of the right ventricle (1), and thus has important clinical implications for injury via myocardial infarction. The goals of this work are to track the structural and functional changes before and after the induction of a myocardial infarction to assess the regenerative capacity of MRL mice versus a non-regenerative control strain (C57Bl/6). Cardiac MR imaging of high spatial and temporal resolution allows non-invasive, longitudinal assessment of cardiac structure and is used in conjunction with finite element modeling to characterize the potential regenerative aptitude of MRL mice.

Methods: For surgical preparation, age matched (10-14 wk) C57Bl/6 (21±1g) and MRL (36±4g) mice were anesthetized with 2 Vol-% isoflurane, intubated and ventilated. Permanent myocardial infarcts were induced by ligation of the left anterior descending coronary artery (LAD) at a position 1-2 mm below the left atrial appendage using 7-0 silk suture.

In vivo NMR murine cardiac imaging was performed on a 7T horizontal-bore MR scanner (Varian, Palo Alto, CA), equipped with a shielded 12 cm bore gradient system (22 G/cm, risetime 300 μs) (Magnex Scientific, Oxon, UK), and a custom designed 19 mm quadrature driven TEM coil. During scanning, mice are anesthetized with 1.5 Vol-% isoflurane, monitored for temperature (34-37°C) and ECG (400-450 BPM). High resolution MRI experiments were conducted using an ECG and respiratory triggered Fast Low Angle Shot (FLASH) Gradient Echo (GE) pulse sequence (α=90°, TE=1.8ms, TR~R-R interval, 5 avgs) employing fractional echo and time series averaging. Seven to nine 1mm thick short axis slices were collected (apex to base) with a FOV of 25mm^2 and data matrix of 128^2 yielding an in-plane resolution of 195μm^2. Images were acquired at a range of time delays relative to the ECG trigger to isolate end-diastolic and end-systolic frames with a temporal resolution of 10ms. Ventricular volumes and ejection fraction were estimated using a prolate spheroidal model. Manual segmentation of cardiac surfaces provided the geometrical data for input to the finite element model.

Results: ECG and respiratory gated MR provide images of high enough spatial and temporal resolution to clearly determine left ventricular chamber volume and wall thickness for end-diastolic and end-systolic phases. Figure 1 a)-c) illustrates mid-ventricular images of MRL mice at day zero (pre-ligation), day 15 (post-ligation) and day 30. Wall thinning is clearly evident at the anterior ventricular free wall. Septal bulging is also visible in coincidence with chamber dilatation. Versus pre-ligation images, day 15 MRL mice present definitive evidence of myocardial infarction and tissue necrosis as summarized in Table 1. Tissue regeneration is visible at day 30 post infarct as indicated by an increased ejection fraction and restored myocardial tissue at the anterior free wall. Figure 1 d)-f) shows finite element models corresponding to the three time points represented in a) through c).

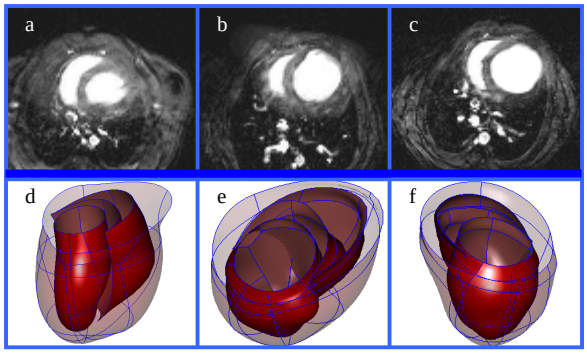

Figure 1. Gated FLASH GE MR images of mid-ventricular MRL mouse heart at a) day zero (pre-ligation), b) day 15, and day 30 post LAD infarct. d) e) f) corresponding finite element models

Table 1 *Indices of Cardiac Function*

	day 0	day 15	day 30
diast. LV volume (μl^3)	47.7	97.9	95.5
ant. wall thick. (mm)	0.87	0.52	0.74
ejection fraction (%)	52.6	16.6	27.6

Discussion: The ability to regenerate myocardial tissue after injury is not common in mammals (2). The predominant response to injury is scar formation. Thus the MRL strain provides a unique and valuable model for understanding the regenerative capacity of the heart on a cellular and tissue level. Characterizing the structural and physiological changes serially in a non-invasive manner is crucial in terms of tracking the healing response through time.

Finite element models have proven to be a powerful tool in understanding cardiac biomechanics and remodeling (3). The integration of geometrical data obtained from MR techniques with finite element schemes allows the construction of parametric models that behave as closely *in silico* as they do *in vivo*. Parameters for the model such as material properties and boundary conditions may be obtained from *ex vivo* experiments, expanding the utility of the models to the prediction of stress and strain within the myocardial wall. Thus, high field fast MRI in conjunction with finite element modeling is capable of characterizing form and function in this novel regeneration model. Initial results suggest the MRL strain of mice has the ability to regenerate myocardial tissue and recover cardiac function after injury.

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