# Combined Manganese-Enhanced MRI and DTI Methods to Assess Post-Myocardial Infarction Molecular and Structural Remodeling

**B. Waghorn[1,2], W. Li[3,4], X. Yu[3,4], and T. C-C. Hu[1,2]**

[1]Department of Radiology, Medical College of Georgia, Augusta, Georgia, United States, [2]Nuclear and Radiological Engineering and Medical Physics Programs, Georgia Institute of Technology, Atlanta, Georgia, United States, [3]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio, United States, [4]Case Center for Imaging Research, Case Western Reserve University, Cleveland, Ohio, United States

## INTRODUCTION

Coronary heart disease (CHD) remains the leading cause of death in the United States, accounting for approximately 26% of the total US deaths in 2006. Myocardial infarction (MI) accounts for approximately half of the CHD cases (1). In the post-MI heart a number of molecular and structural changes are observed, including increased intracellular $Ca^{2+}$ concentrations in the ischemic peri-infarct zones, and myocardial thinning in the infarcted regions. This study uses molecular MRI contrast agent $Mn^{2+}$, known to act as a $Ca^{2+}$ analogue, to assess the molecular changes in $Ca^{2+}$ handling post-MI surgery in a mouse model, while DTI studies were subsequently performed in the excised heart to evaluate myocardial structural changes. Ultimately this study is designed to examine the relationship between indirect $Ca^{2+}$ handling and structural modification during the myocardial remodeling process. Results from this study could provide a multiple diagnostic method for monitoring the salvageability of the peri-infarcted zone.

## METHODS

Magnetic resonance imaging studies were performed in C57Bl/6 mice (n = 14, average weight = 23.8±2.0g, 9-13 weeks old), as previously described (3). The mice were separated into two groups; a control group undergoing no surgery (n = 6) and a myocardial infarction group (n = 8) where the mice underwent surgical procedures to ligate the left anterior descending coronary artery. *In vivo* manganese-enhanced MRI (MEMRI) studies were conducted 8±1 days post-surgery.

**Manganese-Enhanced MRI** MEMRI short axis $T_1$ maps were acquired mid-way through the left ventricle, both pre- and post-280 nmoles/g BW $MnCl_2$ infusion, on a 7T Bruker scanner. A Look-Locker pulse sequence was used, as previously described (4): matrix = 128 x 128; TE/TR = 2.5 ms/10 sec; slice thickness = 1.0 mm; FOV = 3.0 x 3.0 cm; NA = 2; inversion time = 9 ms; average echo interval = 138 ms (determined by the average R-R period before acquisition); number of echo images = 50; average flip angle = 11±1°. A regional analysis was performed in both groups to assess the increase in relaxation rate, ($\Delta R_1 = 1/T_1$ post – $1/T_1$ pre-$MnCl_2$ infusion). Following imaging the mice were sacrificed, and the heart was excised as previously described (5).

**Diffusion Tensor MRI** One day before performing DTI, the post-MI (n = 7) and control (n = 5) fixed hearts were rinsed and suspended in 1X PBS. Diffusion weighted images were acquired on a 9.4T Bruker scanner under room temperature using a spin-echo sequence with a bipolar diffusion gradient. Seven 0.5 mm thick short-axis slices were acquired to cover the whole left ventricle (LV). Imaging parameters were: TE = 34 msec; TR = 2.5 sec; δ = 10 msec; Δ = 20 msec; Diffusion direction = 6; FOV = 1x1 $cm^2$; b = 800 $s/mm^2$; NA = 12; Matrix size = 128x128; Resolution = 78x78 $\mu m^2$.

**DTI Data Processing** Diffusion tensor matrix and the three corresponding eigenvalues were calculated from diffusion-weighted image sets using an in-house MATLAB-based software. Diffusivity map, defined as the average of the three eigenvalues, was normalized to that of the surrounding PBS solution to minimize the variation in diffusivity caused by temperature fluctuation. Fractional anisotropy (FA) maps were generated to quantify diffusion anisotropy. The infarct area was visually identified as the region with significant LV wall thinning. The remaining myocardium was divided into six equal pieces in circumferential direction. The adjacent, peri-infarct area was defined as the two regions neighboring the infarct zone (Fig.1).

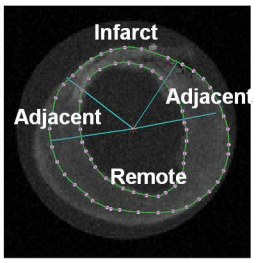


*Figure 1 – Illustration of the DTI based infarct, adjacent and remote zones.*

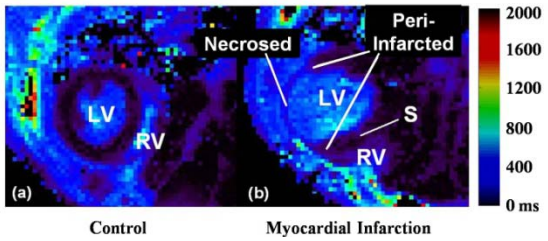


*Figure 2 – Example short-axis mouse heart $T_1$-maps (a) control and (b) MI, 0.2 hrs post-$MnCl_2$ infusion. Left and right ventricles, LV and RV respectively*

**DTI Statistical Analysis** All results were expressed as mean ± SD. Unpaired student's *t*-test was used for intergroup comparison of the parametric variables. A 2-tailed value of $P<0.05$ was considered significant. Two-way ANOVA was performed for comparison of helix and transverse angle from the four groups.

## RESULTS

The infarcted regions of the post-MI myocardium exhibited significant reductions in $Mn^{2+}$ uptake compared to both the healthy, remote tissue, and the myocardial tissue in the control group ($p < 0.05$) (Fig 2). A radially changing uptake in $Mn^{2+}$ was similarly observed for the peri-infarct zones. Compared with the control hearts, a significant decrease in all three diffusion eigenvalues was observed in the infarcted area (Fig. 3). As a result, normalized average diffusivity decreased from 0.57±0.09 to 0.45±0.06 ($P<0.05$). In addition, the infarcted area showed a significantly greater FA value (0.39±0.07 vs 0.28±0.06). Within the infarct heart, the remote and adjacent regions showed increased diffusivity and decreased FA compared with the infarct zone. Helix angles changed continuously from +35 degree at endocardium to -30 degree at epicardium in all groups. Transverse angles were within ±10 degrees (Fig. 4). Two-way ANOVA revealed that myocardial infarction had no significant effects on helix or transverse angles.

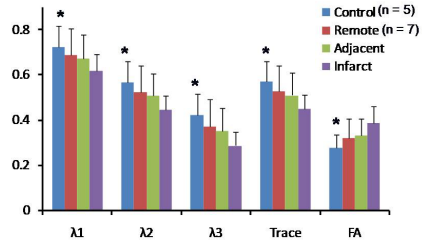


*Figure 3 – Diffusion parameters of the control and infarct hearts. *P<0.05 compared with infarct zone.*

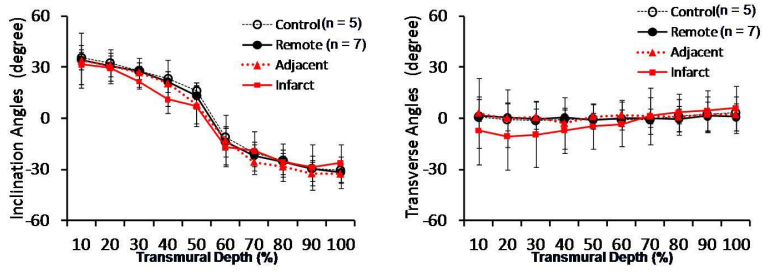


*Figure 4– Transmural course of the inclination and transverse angles. Error bar shows the intra-animal variations.*

## CONCLUSIONS

A decrease in $Mn^{2+}$ uptake was observed for the necrotic, infarcted tissue, as well as the ischemic peri-infarct tissue. This is likely caused by changes in $Ca^{2+}$ handling post-MI, with the contrast agent $Mn^{2+}$ acting as in indirect molecular predictor for $Ca^{2+}$ handing. A decrease in diffusivity and an increase in diffusion anisotropy were observed in the infarct hearts, which is consistent with the literature (6). The underlying pathological process may be the formation of granulation tissue, which was more organized in fiber-like structures. The preserved transmural helix and transverse angles suggest that the global fiber structure for both collagen scaffold and myocytes was well preserved in the hearts with myocardial infarction.

## REFERENCES

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