Ex-Vivo Cardiac Fibre Imaging using Diffusion Tensor MRI and Optical Projection Tomography

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Introduction

Myofibre orientation in the heart strongly influences its ability to function efficiently. This is due to the importance of fibre co-ordination in both mechanical function and electric propagation [1]. During myocardial infarction cardiomyocytes are starved of oxygen and a necrotic region forms that evolves into collagen based scar tissue, altering fibre structure. Knowledge of the fibre morphology in the myocardium would be a useful parameter for prognosis following myocardial infarction. By detecting the preferential movement of water molecules in the myocardium using diffusion tensor imaging (DTI) it is possible to investigate the orientation of muscle fibers in the tissue non-destructively. Optical Projection Tomography (OPT)[2] is an emerging technique that enables imaging of fluorescent reporters within intact tissue by optically clearing the organ and provides complimentary information, such as gene expression, to DTI. This study aims to combine DTI and OPT in the adult heart to reveal structure/function relationships in the various components of the myocardium.

Methods

Myocardial infarction was induced in adult rats by occlusion using suture ligation of the left anterior descending coronary artery for 40mins followed by reperfusion. After seven days hearts were perfused with 150ml of heparinized phosphate buffered saline with high potassium followed by 150ml of 4% formaldehyde solution. The hearts were then stored in formaldehyde for 18hrs followed by PBS solution for 48hrs, changing the solution at 24hrs. Before scanning the hearts were embedded in 1% agarose to prevent motion. The hearts were first imaged using a 9.4T Agilent MRI system with a fast spin echo sequence using two diffusion gradients either side of the inversion pulse with amplitude G = 16G/cm, duration δ = 5ms and separation Δ = 18ms resulting in a b-value of 1.2×10^3s/mm^2. Images were acquired using a 256×256×30 matrix at 0.1×0.1×0.5mm resolution. Analysis of diffusion data was performed using the Camino toolkit[3]. Diffusion tensors were fit using a linear single tensor model and fractional anisotropy and mean diffusivity maps calculated. Following diffusion imaging the hearts were imaged using Optical Projection Tomography. For this the segments were dehydrated in methanol then immersed in BABB (1:2 benzyl alcohol to benzyl benzoate) to clear. Three dimensional images were acquired using 425nm laser illumination at 9µm spatial and 0.45° angular resolution. Data was reconstructed from 800 projections using a filtered back projection algorithm.

Results

In cardiac DTI the primary eigenvector can be taken as the local fibre orientation[4]. Figure 1 shows a comparison of the primary diffusion vectors from a healthy and infarcted rat heart imaged using diffusion MRI. The control heart demonstrates a regular pattern of eigenvector orientation throughout the volume with a high degree of order observed between adjacent voxels, whereas in the infarcted areas the eigenvectors can be seen to lose much of their homogeneity (Fig 1 R insert), yet show a similar pattern in the unaffected myocardium. This is likely to be due to the remodeling of the tissue after cell death where the scar tissue forms. Using a Runge-Kutta probabilistic tractography algorithm[5] the myofibres can be tracked through the myocardium visualizing the fibres in 3D, see figure 2. Figure 3 shows an OPT image with contrast originating from the tissues autofluorescence. Comparing DTI and OPT data will enable structure and function to be assessed using fluorescent labeled cells/tissues as well as gene expression associated with angiogenic and hypertrophic factors with myofibre remodeling following myocardial infarction.

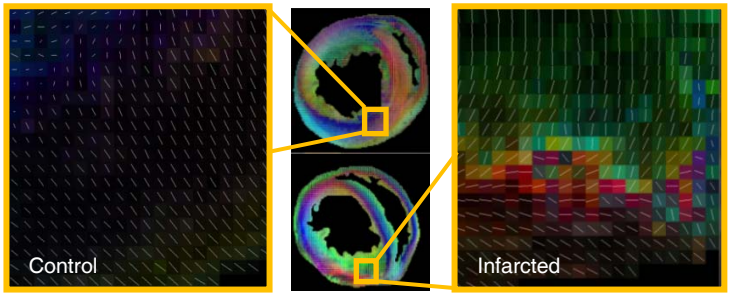

Figure 1. Comparison between infarct (R) and control (L), voxel colour represents direction of primary eigenvector; pixel brightness shows the degree of disorder with surrounding pixels, i.e. bright regions show disorganization; which is typical of infarcted tissue.

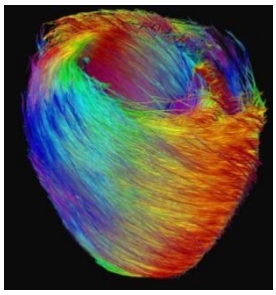

Figure 2. Cardiac tractography showing macroscopic architecture of myofibres

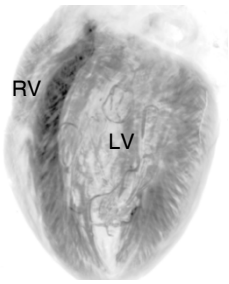

Figure 3. OPT autofluorescence projection, showing high resolution (9µm) morphological detail

Discussion

This preliminary work shows that we are able to assess fiber orientation in control and infarcted rat heart, providing a platform for investigation of disease and novel therapies. Future work will combine and co-register DTI with optical projection tomography data together with vascular casts by computed tomography, to determine the relationship between myocyte orientation or vascular structure and fiber orientation.

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