HIGH-RESOLUTION EX-VIVO MR ANGIOGRAPHY OF THE MURINE HEART USING LANGENDORFF GD-DTPA PERFUSION TECHNIQUE

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Introduction: Murine models of coronary vessel architecture have been widely used to study the heart anatomy and physiology, and to investigate the effects of genetic variants on the cardiac 'vascular tree' [1-2]. In this study we present a simple Magnetic Resonance (MR) based technique suitable for acquiring high resolution ex-vivo angiography data from the excised murine heart.

Methods: Hearts were excised from normal mice and from a genetically modified mouse model developed to study cardiac hypertrophy [3]. Animals were humanely killed by cervical dislocation, the aorta cannulated and retrogradely perfused at constant flow (~ 3ml/min) using a Langendorff perfusion system. A first solution containing 0.75mM $CaCl_2$ was perfused to allow the hearts to contract and clear the blood it contained. Next, 10mM BDM (2,3-butane-dione monoxime), an inhibitor of myofibrillar ATPase was then perfused for 6min to stop the contraction. Finally, a formalin solution containing 0.2ml/100ml Gd-DTPA (Magnevist) was perfused for 15min to fix the sample. The hearts were kept immersed in formalin/Gd-DTPA until imaging. Individual isolated hearts were then placed in an Eppendorf tube containing formalin/Gd-DTPA for MR examination.
Angiograms were obtained by post-processing MR data acquired using a 9.4T Bruker Avance spectrometer and a spoiled 3D gradient echo imaging sequence with TR/TE/φ=50/5.3/40°. The field of view (FOV) was 15mm and an imaging matrix of 256x256x256 was employed giving isotropic voxels with a size of 59µm. The raw data was acquired using 20 signal averages. Individual axial slices were processed using the Analyze™ (Biomedical Imaging Resource, Mayo Clinic) software package to remove regions of image hyper-intensity arising in voxels corresponding to both the ventricles and the solution surrounding the heart. The Analyze™ package was subsequently used to generate the angiograms shown in Figure 1.

Results: Acquiring MR data from murine hearts prepared using a Langendorff heart perfusion apparatus together with an MR contrast agent (Gd-DTPA) results in the cardiac blood vessels showing relative hyper-intensity. This has allowed the calculation of angiograms showing at least three levels of vessel branching within the vascular tree (Figure 1).

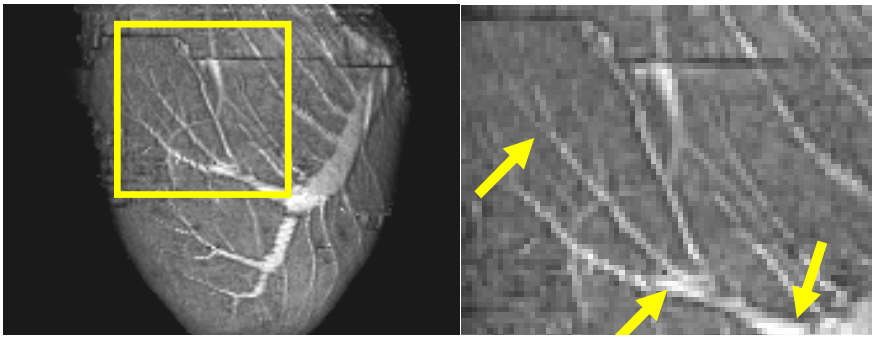

Figure 1. Volume composted images obtained from the heart of a normal mouse (FOV=15mm).
Right hand image shows an enlargement corresponding to the area indicated by the box on the image on the left.
Arrows indicate successive vessel branches.

Discussion: The methodology outlined in this presentation is considerably easier to implement than a previously published technique [1-2] for studying vascular architecture that relies on the injection of resin to generate vascular casts.
Our preliminary results (Figure 1.) demonstrate the potential for our MR based method to depict epicardial vessel architecture with high spatial resolution. Individual vessels with a diameter of approximately 120µm can be detected in the image data.
Alterations in epicardial vessel architecture caused by pathology or genetic variation/modification occurring in vessels of this size (or greater) could therefore become apparent using the technique presented here.

References: [1] Icardo J. et al. J. Anat. 2001; 199:473-482. [2] Fernandez B. et al. J. Anat. 2008; 212:12-18. [3] Ainscough J. et al. Cardiovasc Res. 2008 in press.