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Original research

Mapping cardiac microstructure of rabbit heart in different mechanical states by high resolution diffusion tensor imaging: A proof-of-principle study

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ABSTRACT

Myocardial microstructure and its macroscopic materialisation are fundamental to the function of the heart. Despite this importance, characterisation of cellular features at the organ level remains challenging, and a unifying description of the structure of the heart is still outstanding. Here, we optimised diffusion tensor imaging data to acquire high quality data in *ex vivo* rabbit hearts in slack and contractured states, approximating diastolic and systolic conditions. The data were analysed with a suite of methods that focused on different aspects of the myocardium. In the slack heart, we observed a similar transmural gradient in helix angle of the primary eigenvector of up to 23.6°/mm in the left ventricle and 24.2°/mm in the right ventricle. In the contractured heart, the same transmural gradient remained largely linear, but was offset by up to +49.9° in the left ventricle. In the right ventricle, there was an increase in the transmural gradient to 31.2°/mm and an offset of up to +39.0°. The application of tractography based on each eigenvector enabled visualisation of streamlines that depict cardiomyocyte and sheetlet organisation over large distances. We observed multiple V- and N-shaped sheetlet arrangements throughout the myocardium, and insertion of sheetlets at the intersection of the left and right ventricle. This study integrates several complementary techniques to visualise and quantify the heart's microstructure, projecting parameter representations across different length scales. This represents a step towards a more comprehensive characterisation of myocardial microstructure at the whole organ level.

1. Introduction

The heart's pump function depends on cardiomyocyte activity. These cells occupy the majority of the ventricular volume, and are organised in a highly structured three-dimensional (3D) pattern (Kohl, 2003). The two perhaps most prominent macroscopic descriptors are (i) locally prevailing cardiomyocyte orientation, often referred to as 'fibre orientation' (Holmes et al., 2000), and (ii) laminar structures of laterally interconnected myocyte layers known as 'sheetlets' (Hales et al., 2012; Sands et al., 2005). The organisation and precise distribution of cardiac cells play important roles in the complex electromechanical properties in the heart (Katz and Katz, 1989). During systole, a combination of cardiomyocyte contraction and shear between layers of cardiomyocytes leads to longitudinal shortening of the ventricle in the form of an atrio-ventricular valve plane shift, radial thickening of the ventricle wall predominantly in a centripetal direction, and torsion of the ventricles by apical rotation relative to the base of the heart (Axel et al., 2014). The 3D organisation of fibres and sheetlets has been shown to influence both mechanical contraction (Waldman et al., 1988) and electrical propagation (Kanai and Salama, 1995) in the heart, and has been implicated in ventricular hypertrophy (Ferreira et al., 2014; McGill et al., 2012; Tseng et al., 2006), infarct remodelling (Wickline et al., 1992) and arrhythmia (Chen et al., 1993). Accurate characterisation of the myocardial

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architecture is therefore fundamental for understanding its role in health and disease.

Traditional histo-anatomical characterisation methods, such as gross anatomical dissection or histology, have laid the foundation for our current understanding of cardiac structure (Streeter Jr, 1979). While the presence of helical fibre alignment in the heart has been known for centuries (Lower, 1669), more recent studies have shown that fibre orientation changes from a left-handed helix at the epicardium to a right-handed helix in the endocardium (Streeter et al., 1969). Due to excellent resolution and cell-type specificity, histological methods form a 'gold standard' for tissue characterisation. At the same time, these techniques are associated with tissue distortion and disruption during sample preparation, limited spatial coverage, and non-trivial challenges during reconstruction of whole heart two-dimensional (2D) data stacks into 3D volumes (Burton et al., 2014; Plank et al., 2009).

Other tissue imaging methods are available. These include anatomical magnetic resonance imaging (MRI) (Bernus et al., 2015; Burton et al., 2006) and X-ray computed tomography (Ni et al., 2013), with excellent sample coverage suitable for whole organ studies, but at a resolution in the 10^{-5} m region, they do not yet resolve cellular features. Polarised light imaging also offers good coverage, but resolution is limited to the 10^{-5} m range, and fibre orientation estimates are sensitive to the elevation angle of the cells (Jouk et al., 2007). Scanning electron microscopy (LeGrice et al., 1995) and confocal microscopy (Young et al., 1998), in contrast, provide the highest resolution of 10^{-9} m and better, but are limited in field-of-view.

Diffusion tensor imaging (DTI) (Basser et al., 1994) enables 3D structural mapping of whole hearts. While the imaging resolution is on the order of 10^{-4} m, the technique is sensitive to the microscopic diffusion of water in the 10^{-6} m range, and its interactions with the cellular environment. This facilitates assessment of the 3D arrangement of cardiac fibres and sheetlets (Helm et al., 2005; Scollan et al., 1998). In DTI, the diffusion of water is modelled as a 3D tensor. The lengths and orientations of the three mutually orthogonal major axes (longest, intermediate and shortest) of this tensor are described respectively by its eigenvalues and eigenvectors. The primary, secondary and tertiary eigenvectors ($\mathbf{v_1}$, $\mathbf{v_2}$, $\mathbf{v_3}$) are understood to correspond to the fibre long-axis, the fibre-normal/in-sheet and the sheetlet-normal directions respectively, of cells in myocardial laminae (Helm et al., 2005; Scollan et al., 1998), as validated by histology (Holmes et al., 2000; Hsu et al., 1998; Kung et al., 2011; Scollan et al., 1998).

Several studies have utilised DTI to quantitatively investigate rabbit heart structure. Early work characterised fibre and sheetlet orientations in healthy rabbit hearts, and linked $\mathbf{v_3}$ to the orientation of the ventricular sheetlets (Scollan et al., 2000). Rabbit hearts have also been used in important histological validation studies of DTI, which clarified the correspondence of the diffusion signal to the underlying microstructure (Holmes et al., 2000; Scollan et al., 1998). In these studies, 2D fast spin echo DTI data were acquired at an imaging resolution of 156 × 312 × 2000 μm. More recent work investigated how well the fibre orientation is conserved across species in mice, rabbit and sheep (Healy et al., 2011). Using a 3D spin echo acquisition at 250 μm isotropic resolution, the study focused only on the helix angle of $\mathbf{v_1}$ in the left ventricle.

Improvements in magnet and gradient system hardware, as well as data acquisition protocols, now allow one to acquire high quality DTI data at comparatively high isotropic resolution in the isolated fixed heart. Here, we combine a state-of-the-art preclinical MRI system with optimised 3D spin echo DTI acquisition to improve the signal-to-noise ratio, supporting an isotropic imaging resolution of 200 μm in *ex vivo* rabbit hearts. Both $\mathbf{v_1}$ and $\mathbf{v_3}$ are assessed and quantified in multiple wall regions and heights in both the left ventricle (LV) and right ventricle (RV). Superquadric glyphs are used to aid voxel-wise display of tensors, transmural angle profiles are quantified regionally, and global tractography is employed to help visualise macroscopic distributions of fibres and sheetlet alignments. The study (i) uses multiple approaches to augment our understanding of myocardial structure in rabbit hearts, and (ii) investigates structural differences between the diastolic (slack) and systolic (contracture) states.

2. Materials and methods

2.1. Sample preparation

All experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986 (UK). Hearts were isolated from two male New Zealand White rabbits after induction of terminal anaesthesia (pentobarbitone under Schedule 1 protocol). Isolated hearts were swiftly cannulated and perfused in constant pressure Langendorff mode for 5 min with normal physiological saline (in mM: NaCl 125, $NaHCO_3$ 25, KCl 5.4, NaH_2PO_4 1.2, $MgCl_2$ 1, Glucose 5.5, $CaCl_2$ 1.8, pH to 7.4 with NaOH and oxygenated with 95% O2/5% CO2) containing heparin (5 IU/mL). The first heart was arrested using physiological saline with elevated potassium (20 mM KCl) to induce diastolic arrest, while the second heart was exposed to lithium-replacement of sodium to induce contracture (in mM: LiCl 125, KCl 5, $MgCl_2$ 1, HEPES 10, Glucose 11, $CaCl_2$ 2.5, pH 7.4) (Burton et al., 2006). Hearts were then perfused via the aorta with isosmotic Karnovsky's fixative (300 ± 10 mOsm, Solmedia UK) while immersed in fixative to avoid ingress of air into cardiac chambers. Following a fixation period of 4 days, hearts were placed in fixative containing 2 mM gadolinium (Gd) complex Prohance (Bracco, MN, USA). Prior to imaging, the hearts were washed in PBS + 2 mM Gd, and embedded in 1% low melting point agarose gel made from PBS + 2 mM Gd.

2.2. Data acquisition

Non-selective 3D spin echo DTI data were acquired on a 9.4 T preclinical MRI scanner (Agilent, CA, USA) with a shielded gradient system (max gradient strength = 1 T/m, rise time = 130 μs), and transmit/receive quadrature coil (inner diameter = 42 mm; Rapid Biomedical, Rimpar, Germany). Acquisition parameters were: repetition time = 250 m, echo time = 10 m, field-of-view = 43.2 × 28.8 × 28.8 mm, matrix size = 216 × 144 × 144, resolution = 200 × 200 × 200 μm, number of non-diffusion-weighted (DW) images = 3, number of DW directions = 12 (Cook et al., 2007), diffusion duration (δ) = 2 m, diffusion time (Δ) = 5.5 m, b-value = 1000 s/mm^2, acquisition time = 21:36 h. Based on these diffusion times and assuming unrestricted diffusion, the root mean squared displacement in tissue with apparent diffusivities ranging from 1.0–1.5 × 10^{-3} mm^2/s would have ranged from 3.1 to 3.8 μm (Latt et al., 2007). Sample temperature variations, arising from warming of the gradient coils during the application of diffusion gradients, were minimised by interleaving DW and non-DW scans. The receiver gain was increased by 10 dB during the DW scans to increase the signal-to-noise ratio, while remaining within the dynamic range of the receiver. The amplified DW signals as a result of the higher gain were normalised by separate non-localised gradient echo data acquired at the respective receiver gains. The gradient system was calibrated prior to the experiment to minimise directional bias in the diffusion measurements (Teh et al., 2016).