

Cardiac Purkinje Fiber Imaging: the first instance of in situ visualization of the conduction path using MR microscopy

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Introduction

The impulse-conducting system that coordinates the cardiac cycle is a feature of a highly heterogeneous structure in the sense that the anatomical make-up of the system has intrinsic differences in cell size, cell type distribution, complex electromechanical properties, and activation sequence [1]. The properly integrated function of the system for synchronous and reliable delivery of the electrical signal appears necessarily to require the well-orchestrated interplay of the heterogeneous components. Thus, detailed insight into the anatomy of the conduction system would be of great importance for the understanding of the homogeneous cardiac performance arising from well-coordinated electro-mechanical activity and further for the investigation of cardiac function in a (an) normal and abnormal state [2, 3]. To date, the techniques most commonly employed to study the cardiac conduction system include mapping from the histological serial sectioning of a few micrometer's thickness, micro-dissection, endocardial staining, and direct ink injections [4]. However, those use intrinsically destructive procedures and have shown limitations when trying to trace the individual bundles or branches of the conduction system to show varying pathways. Moreover, three dimensional reconstruction of the Purkinje fiber network using histological data is very difficult, requiring much labor and time. Consequently, the need for non-invasive data suitable to describe the conduction system and the anatomical features of the heart makes magnetic resonance imaging (MRI) an especially promising modality. In this experimental study, we performed high resolution MR imaging using a 17.6 T magnet to demonstrate the conduction pathways as well as anatomical details of isolated rabbit hearts.

Materials and Methods

Isolated heart preparation: Isolated perfused hearts (n = 5) of New Zealand White male rabbits (3 ~ 5 kg) were prepared according to the animal protocol approved by the UF Institutional Animal Care and Use Committee. The isolated hearts were fixed in situ during an intravascular formalin-perfusion fixation procedure that was carefully standardized. And the fixed hearts were kept in a refrigerator until MR imaging experiments.

MRI: MR experiments of the isolated hearts were performed on a 17.6 T / 89 mm vertical wide-bore magnet (Bruker Instrument spectrometer and console, Billerica, MA). The RF coil used for the in vitro imaging was an Alderman-Grant birdcage coil, diameter = 25 mm, length = 35 cm. The temperature in the magnet was maintained at 19 - 20°C. Three dimensional high resolution MR image data were collected using a fast gradient echo pulse sequence, achieving a voxel resolution of 35.2 $\mu\text{m} \times 35.2 \mu\text{m} \times 82 \mu\text{m}$ with a matrix size = 710 x 710 x 256 in the field of view of 25 mm x 25 mm x 21 mm. Imaging parameters implementing relatively T2*-weighting were TR = 150 ms, TE = 18.5 ms, 1 average, sampling bandwidth = 20 k. The pilot images with three orthogonal planes were collected to check whether or not the isolated heart imbedded in the dense FC-43 solution moved during the relatively long scans.

Data Analysis: Volume rendering of the 3D MR data sets were performed using ImageJ (ver. 1.31, <http://rsbweb.nih.gov/ij/>) in the transverse, sagittal, and coronal directions for the purpose of tracing from the atrioventricular connecting bundle and its left and right branches in the ventricular septal muscle down to the termination of the conduction path, the so-called Purkinje-ventricular junction. Anatomical analyses of the rendered images were conducted based on optical images and literature.

Results and Discussion

High resolution MRI can be used to visualize free-running Purkinje fiber network [3]. To the best of our knowledge, this is the first instance of in situ non-invasive conduction path visualization from bundle branches to Purkinje-ventricular junction using MR microscopy. The volume rendered images from the original 3D MR data, achieving a 35 μm in-plane resolution and generating an adequate T2*-weighted image contrast, made it possible to non-invasively and reproducibly trace the conduction paths in the left and right ventricles, as well as to describe the micro-anatomical make-up of the whole heart. Since tendinous fiber-like cords observed in the left and right ventricular cavities (Fig.1, left and Fig. 2), called as the free-running Purkinje fibers, are known as parts of the conduction path [2, 3], we could follow superiorly back the Purkinje fibers into the right and left bundle branches forming fascicular structures (Fig.1, middle) and then to the connecting bundle (Fig.1, right). The anatomical landmarks were used to enhance our confidence in the interpretation that the observed structures (yellow arrows) represent the conduction paths. Histochemistry taining is currently underway for the definitive proof. The free-running Purkinje fibers form a reticular dense polygonal net in the ventricular cavities and are connected with the conical masses of the anterior or posterior papillary muscles (Fig. 2). Seen overall, there appears to exist discrete regions that have denser Purkinje fiber network in the LV cavity, in particular around the papillary muscles and near to the apex of the heart. The Purkinje fibers appear to enter into the subendocardium for ventricular depolarization. MR characteristics of the Purkinje fibers and the detailed Purkinje-ventricular termination revealed by MR microscopy are subject of future study. Papillary muscles that the free-running Purkinje fibers are attached to will be harvested to gain the goal.

Conclusion

The MR volume rendered images with appropriate views and cuts could demonstrate non-invasively the conduction paths observed in previously histological studies. Further, those images appear to describe efficiently detailed or integrative features difficult to visualize with destructive techniques because sectioning in the inappropriate direction often resulted in the loss of ability to trace the desired bundle and branches. When taking into account labor and time required for sectioning of the whole heart, the intrinsic capability of MR as an efficient and integrative tool is very attractive and promising to elucidate the anatomy-function relation and to develop a working animal model, leading ultimately to application to humans.

References: 1. A. Katz and P. Katz Circulation 1989; 79: 712-7. 2. A. Ansari et al. Anat Rec 1999; 254: 92-7. 3. C. Goodyer et al. Conf Proc IEEE Eng Med Biol Soc 2007: 147-51. 4. R. Burton et al. Ann N Y Acad Sci 2006; 1080: 301-19.

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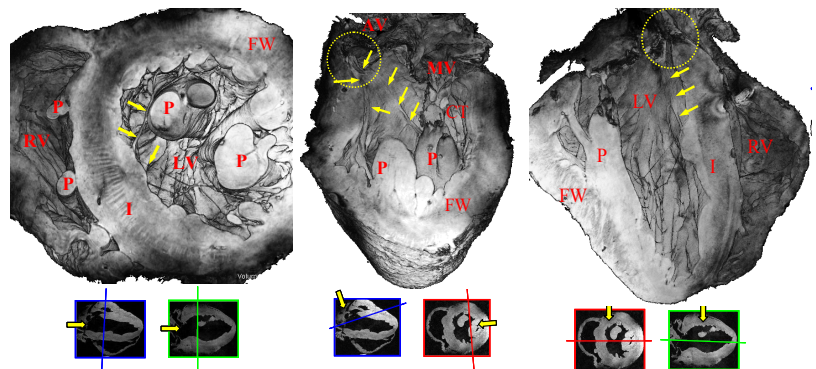


Fig.1 Volume rendered 3D MR images to visualize the conduction path from the Purkinje fiber network (left), the left bundle branches (middle, yellow circle), and to branching into left and right bundle (right, yellow circle). Images were harvested with appropriate views and cuts. Lines and arrows in the red, green and blue boxes indicate where sectioning occurred and viewer was located. Yellow arrows follow back the Purkinje fibers. AV: leaflets of the aortic valve, MV: leaflets of the mitral valve, CT: chordae tendinae, FW: free wall

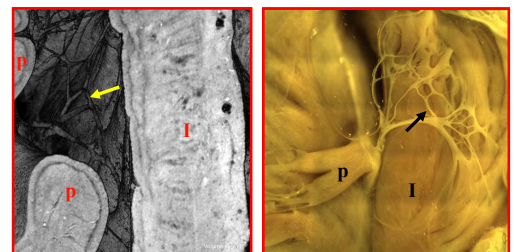


Fig. 2 Polyagonal free-running Purkinje fiber network (arrows) in the LV cavity. A magnified volume rendered MR image (left) and an optical image of Acetylcholine esterase staining (right, unfolded). I: ventricular interseptum, P: papillary muscle.