Non-invasive Monitoring of Alterations in Rabbit Hearts with Aging Using MR Microscopy

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Introduction

It is well-known that morphological changes occur in the heart as a result of aging. Loss of myocytes with subsequent compensatory hypertrophy of the remaining cells and interstitial fibrosis are common hallmarks observed [1, 2]. The cardiac conduction system is also subject to alterations with aging due to the calcification within the cardiac skeleton [3]. Biopsy is an accurate and informative means to delineate the age-related changes in the heart. However, it is destructive and requires intensive labor, often with severe complications for 3D reconstruction. Also, in discrete focal lesions, one runs the risk of missing the pathology due to undersampling. The aim of this experimental study was to investigate non-invasively the morphological changes in aged heart using MR microscopy (MRM) and high angular resolution diffusion microscopy (HARDM). Purkinje fiber networks in the left ventricular cavity of young and old rabbit hearts were compared using 3D MRM and HARDM.

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 approved animal protocol. Young hearts (n = 2) were 6 month ~ 1 year old. Old hearts were 3 ~ 4 years old. The isolated hearts were fixed in situ during an intravascular formalin-perfusion fixation procedure that was carefully standardized. Fixed hearts were kept in a refrigerator until MR imaging experiments. Forty eight hours prior to the start of imaging, hearts were transferred to phosphate buffered saline solution to wash out residual fixative.

MRI: MR experiments of the isolated hearts were performed on a 17.6 T / 89 mm vertical wide-bore magnet (Bruker Instruments, 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 μm x 35.2 μm x 82 μm 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. For direct geometrical matching with 3D imaging, the subsequent HARDI of 21 directions was performed using a standard PGSE pulse sequence, achieving an isotropic in-plane resolution of 50 μm with a transverse slice of 500 μm. Diffusion sensitizing factor (b-value) was 1000 s/mm^2 using Δ = 13.4 ms and δ = 1.8 ms. Imaging parameters were TR = 3000 ms, TE = 25.1 ms, 1 average. The pilot images with three orthogonal planes were collected during the experiment to determine if the isolated heart imbedded in the dense FC-43 solution moved during long scans (~ 24 hours).

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. Anatomical analyses of the rendered images were conducted. The tensor processing of HARDM data sets was performed using fanDTasia™ [4].

Results and Discussion

Aging in rabbit heart appears to show a significant increase interseptal thickness (~ 22.6 %; $p<0.05$) (Fig.1). Volume rendered transverse images of the apical half suggest that the Purkinje fiber network in LV cavity is significantly altered with aging (Fig. 1). A young heart appears to have a significantly complex polygonal arrangement of free running Purkinje fibers. Hearts from older animals have free-running Purkinje fibers that are thinner, and have a decreased polygonal network in the ventricular cavity. We speculate that the alteration of the free-running Purkinje fiber network may correlate with stiffened Purkinje fibers due to increased collagen content and higher left ventricular outflow tract velocity which have been observed in aged heart [3].

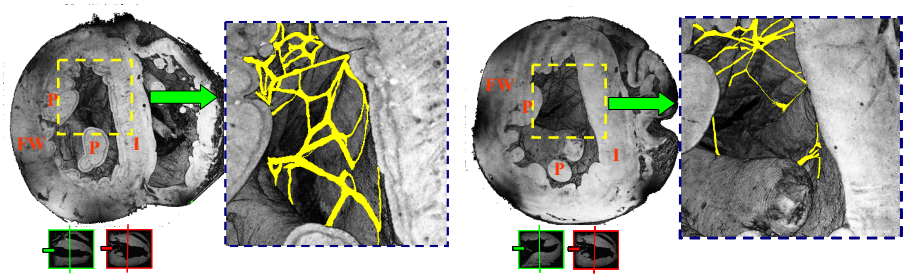

Fig. 1 Volume rendered transverse images and manual segmentation (magnified) of the free-running Purkinje fiber network in the LV of a young rabbit heart (left) and an old rabbit heart (right). Lines and arrows in the green and red boxes indicate where sectioning occurred and viewer was located. Interseptal thickness is approximately 3.93 mm (young) and 4.82 mm (old). I: ventricular interseptum, P: papillary muscle, FW: free wall

From HARDM, aging appears to be associated with changes in water diffusity, which may arise from cellular hypertrophy (data not shown). Interstitial fibrosis that occurs in the aged heart may also be responsible for the increase in the fast component of the multiexponential diffusion signal. A tensor component map of a young heart (D_{yz}) shows a conspicuous stripe pattern in the interseptum and freewall (Fig. 2, left). In contrast, this region is significantly reduced in hearts from older animals (Fig.2, right). A volume rendered image of intersepum shows that the stripe pattern has a longuitudinal direction and appears to exist in the basal half of the LV.

Conclusion

Change in the free-running Purkinje fiber network with aging could be non-invasively monitored using MR microscopy. In particular, these results demonstrate that combined analysis of the two MR modalities (MRM & HARDM) may be a powerful tool to understand and monitor alterations in the cardiac conduction network that occur as a function of age.

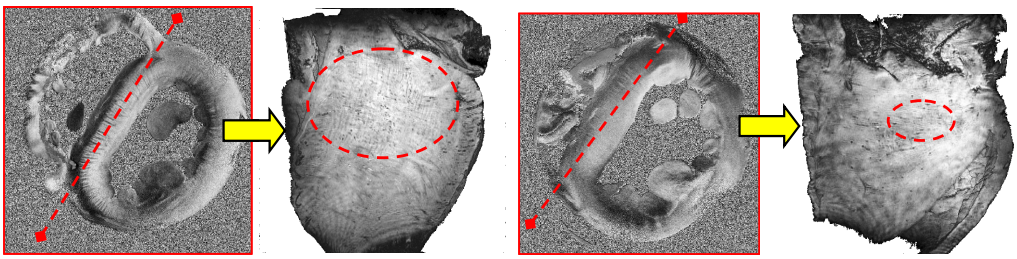

Fig. 2 Tensor component (Dyz) map from HARDI and a volume rendered image of the interseptum of a young rabbit heart (left) and an old rabbit heart (right). Dotted lines (red) indicate where sectioning occurred and dotted circles confine regions where the stripe pattern is observed.

References:
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