First-Order Mapping of Epicardial Elastin Network in Porcine Hearts

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Statement of Purpose: Heart disease is the leading cause of death in the United States each year. To better understand the global heart contraction and relaxation, it is essential to obtain the knowledge in heart muscle organization, and how the complex multilayered helical architecture being mediated by 3D cardiac ECM that spans from epicardium, to myocardium, to endocardium. However, the majority of the previous research on ECM has mainly focused on collagen in heart, while only a few researches have been done to study elastin, another important component in the heart ECM [1-2]. In this study, we aim to delineate and quantify 3D elastin network in epicardium of the porcine left ventricle.

Methods: Porcine hearts (~6 months old) were obtained from a local abattoir. To acquire the 3D image of the elastin network, epicardium samples were dissected from top, middle and bottom sections of the left ventricle in both the anterior and posterior locations. The epicardium samples were then further processed for Laser scanning confocal microscopy (LSCM). 3D elastin network was imaged under red channel (elastin autofluorescence, CY3, Ex = 543 nm) with z-stack imaging technique. The 3D z-stack images cover a thickness range from 40 to 70 μm at ~1 μm intervals. ImageJ was then used to create the 3D projection of the first 15 μm thick layer, the last 15 μm layer, and the full thickness of image stack. Elastin fiber orientation and distribution in different layers were then quantified using OrientationJ plugin. The cross-sections of the epicardium were also prepared for Movat's pentachrome staining for histological validation. In-house custom written software was also used to reveal other 3D features of the epicardial elastin network.

Results: By means of 3D LSCM, we were able to create a first-order mapping of the elastin network in the epicardium of the left ventricle. Elastin fibers were found to be abundant in the epicardium and exhibit certain patterns, which were highly correlated to their anatomical location in the left ventricle. The elastin fiber orientation and fiber alignment were found to often take one pattern/orientation at the surface layer, but change to another pattern/orientation when reaching to the deep layer. As an example, Figure 1 showed an epicardial elastin fiber pattern observed in the left ventricle apex region, in which we noticed a 3D meshwork in the surface layer and a highly oriented wave elastin fiber arrangement in the deep layer (Fig. 1). Other 3D parametric analyses have also been performed in this study.

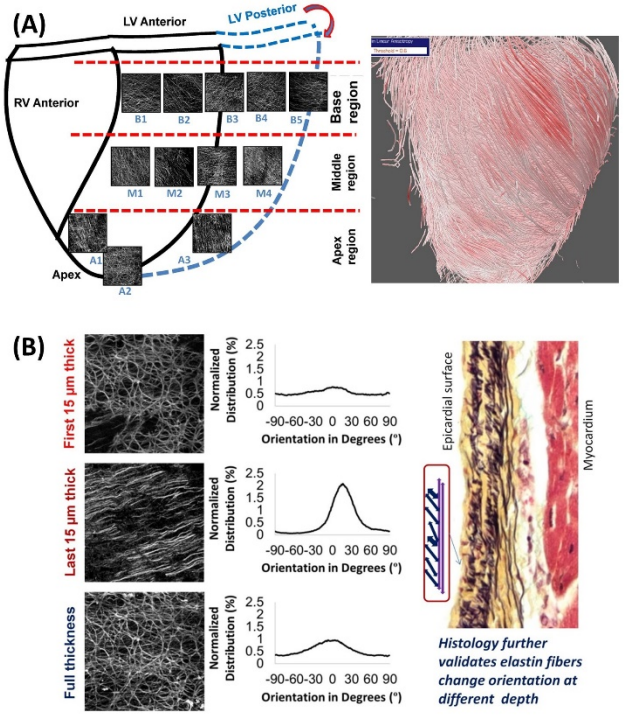

Figure 1. (A) Location-dependence and (B) depth-dependence of epicardial elastin network.

Conclusions: Our study produced a first-order mapping of the epicardial elastin network in the left ventricle. We found that the elastin network showed certain patterns that were highly correlated to their anatomic locations. Besides the location variation, epicardial elastin network also showed a 3D structural alteration from the surface layer to the deep layer. Overall, the observed elastin fiber pattern, fiber orientation, and fiber alignment might serve certain mechanical function that helps coordinating the heart contraction and relaxation. We have further characterized the biaxial mechanical properties of the porcine epicardial layer in order to reveal the correlation between the epicardial elastin network and the epicardial biomechanical behavior. Future work will be focusing on understanding the structural-functional relationship of this 3D elastin design in the left ventricle.

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References:

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