Quantitative Image Analysis and 3-D Digital Reconstruction of Aortic Valve Leaflet

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Abstract

Current efforts in tissue engineering center on designing a viable replacement valve as an alternative to the existing non-viable mechanical and biological prosthetics. Compared with existing prosthetics, this valve would offer better longevity and biocompatibility. To design and construct such a valve, a detailed understanding of the microstructure of the native porcine valve must first be acquired. Histological sections of the right coronary leaflet of the aortic valve, taken along the circumferential direction, were first digitally imaged using bright field microscopy. Then, the slides were scanned individually and digitally stacked to construct a 3-D volumetric rending of the entire leaflet. The results of these imaging techniques will allow researchers to visualize local variations within the leaflet in terms of cell count, cellular layer thickness, and structural protein composition. An inferred understanding of the function behind the structure will help researchers emulate the performance of the aortic valve in the tissue-engineered prosthetics.

Introduction

The aortic valve opens and closes approximately $3x10^9$ times during the lifetime of an average person, thus subjecting the valve to a number deteriorating conditions, such as stenosis from calcification, regurgitative leakage, bacterial infections, and congenital defects. Improper functioning of the valves could lead to abnormal cardiac output, ventricular hypertrophy, and heart failure, making surgical replacement of the damaged valve necessary. Of the 95,000 annual valve replacement surgeries performed in the U.S., 63% are aortic valve replacements.

Tissue engineered (TE) valves offer the following advantages over non-viable mechanical and biological prosthetic: availability, customizability, durability, and bio-compatiblability. The TE valve must adequately mimic the performance of the native aortic valve. Therefore, the structure of the native valve must be understood and used to serve as the standard for the TE valve design.

Research into the structure of the aortic valve has revealed that each leaflet is composed of three cell layers: fibrosa, spongiosa, and ventricularis. The collagenous fibrosa faces the lumen of the aorta. The spongiosa, a middle, non-load-carrying layer, is composed mainly of glycosaminoglycans (GAGs). The layer facing the inside of the ventricle, ventricularis, is composed of elastin and collagen. This study set out to quantify the cellular and structural protein distributions as well as any local variations that exist within each of the three layers. A second component of the project involves constructing a 3-D representation of the leaflet to allow researchers to visualize such local variations.

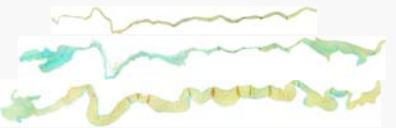
Method

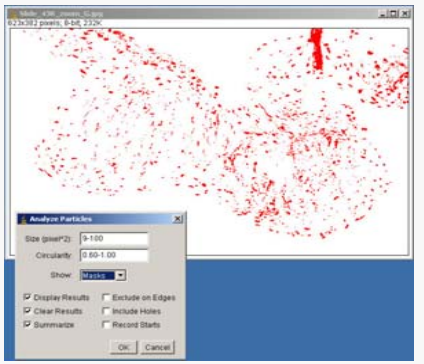
Histology: 5μm thick circumferential slices fixed in formalin stained with Movat's pentachrome

Image Acquisition: for quantification, 17 slices, spaced 90 μm apart, were digitally captured using bright field microscopy at 20x and montaged. For 3D reconstruction: 50 slices, spaced 10-15 μm apart were scanned individually using slide scanner

Image Analysis: layer separation, cytometry/particle analysis, thresholding, and protein content by area ratio measurements conducted in Image J. Layer thickness measurements performed in Metamorph.

3D Reconstruction: Digitally aligned and stacked



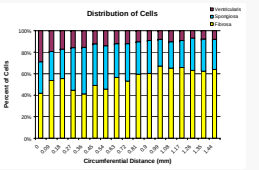


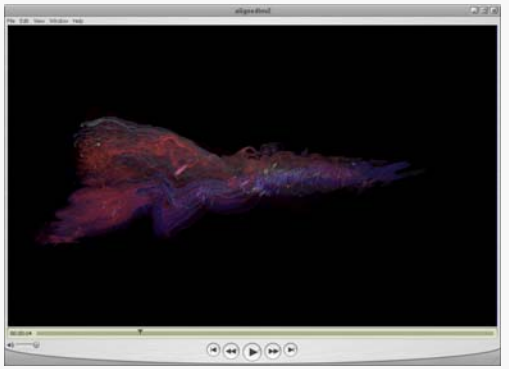
Results

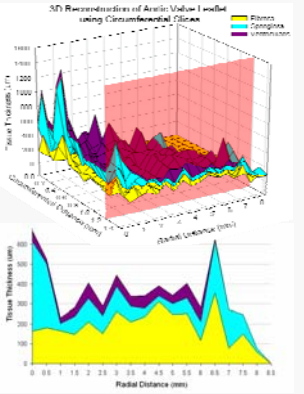
Approximately 15% of the leaflet has been quantified and digitized with the following results:

Collagen and elastin content: 48.2+6% collagen area in fibrosa, 54.3+6% collagen area and 39.3+5% elastin in ventricularis. These results must be compared with data published in literature, which are in unites of weight percent.

Average layer thickness: Fibrosa: 150-230 μm (~100-350 μm), Spongiosa: 110-200 μm (~70-250 μm), and Ventricularis: 40-60 μm (~50-150 μm)







Future Work

- Preliminary results call for completion of quantification and digital reconstruction of the entire leaflet
- Statistical comparison of quantification results with published values
- Explore other imaging techniques (fluorescent microscopy, X-ray, ultrasound, acoustic microscopy, SEM)
- Construct a 3D representation containing quantitative information
- Use 3D reconstruction to simulate and visualize dynamic response to applied load

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