NC STATE UNIVERSITY

Mesostructural changes of heart valve tissue during collagenase degradation

Tyler Ash and Hsiao-Ying Shadow Huang
Mechanical and Aerospace Engineering Department, North Carolina State University, Raleigh, NC

Introduction and Background

Current Knowledge

- Valvular interstitial Cells (VICs) produce matrix metalloproteinases (MMPs) to catabolize damaged collagen fibers and repair tissue, but they can also cause collagen depletion and alter material properties.
- Collagen degradation affects cellular regulations controlled by VICs, and it is related to heart valve diseases.
- Strain inhibits degradation of collagen at a fibrillar level, previous research indicated that physiological strain ratio also had an inhibiting effect on reduction in mechanical properties compared to equibiaxial strain

Current Limitations

- The effects of MMP degradation on the extracellular matrix (ECM) at a local meso-structural level, and the relation with strain state is unknown.

Objectives and Approaches

- An approach to understand and quantify **enzymatic degradation** of collagen fibers is performed
- Porcine aortic valves are immersed in PBS or 0.5 mg/mL collagenase solution to simulate MMPs
- Multiphoton Second Harmonic Generation (SHG) imaging of collagen is performed during the degradation process at 30 min intervals for 180 min using Zeiss LSM 7 MP microscope

Methods and Results

Changes in ECM during Degradation

- The image stacks are analyzed in ImageJ and Matlab to determine the changes in layer thickness, fiber organization, and amount of collagen
- Pixel intensity histogram skewness is used as a depth independent measure of fiber concentration
- Fast Fourier Transform (FFT) is performed then power spectrum analysis to fit a gaussian model to the angular data to quantify organization

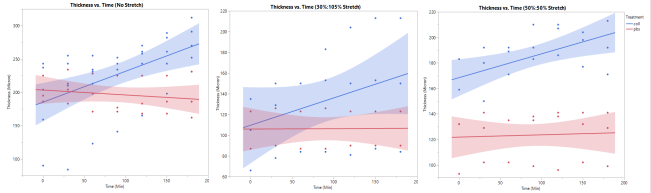

Fig. 1: Plots of changing thickness over time, Using the slopes of regression lines of the collagenase treated data(0.46, 0.26, 0.18), it appears that strain tissues do inhibit this degradation induced swelling

- Using phantom images (**Fig. 2)** of known fiber densities, skew is confirmed to predict density regardless of depth

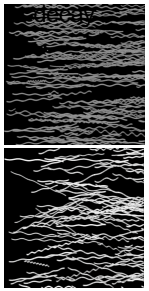

Table 1, Validating skew as measure of fiber density

Skew				
-	Density			
Brightness	60	120	180	240
Reduced	2.1398	1.1202	0.6382	0.3514
Full	2.1166	1.2514	0.824	0.3682

Fig. 2: phantom images for model verification

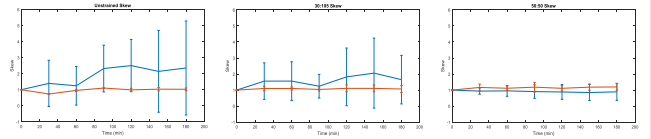

Fig. 3: Skewness of the pixel intensity histogram confirms that degradation appears to be inhibited by strain, but also does not indicate a preference for physiological strain ratio

Discussion and Conclusion

Effects of collagenase degradation of structure of Aortic Valve ECM

- Amount of collagen present decreases on overage based on the skewness histogram,
- Alignment and structure of collagen do not consistently show significant changes over time based on FFT analysis

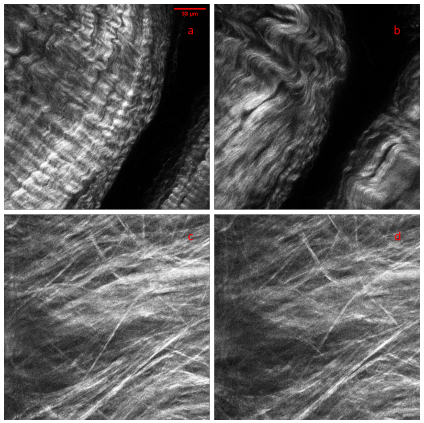

Fig. 4: After Unstrained (a,b) and 50:50% stretch (c,d) images before (a,c) and after(b,d) collagenase treatment, exemplifying the loosening of fiber structures that can occur

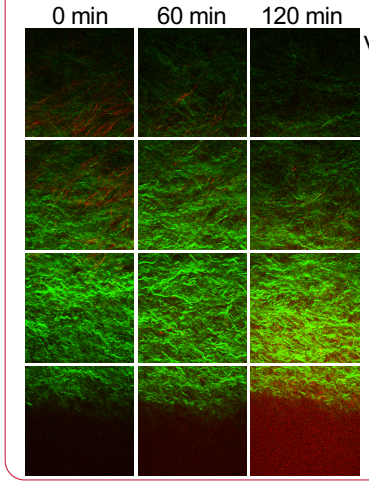

Fig. 5: MP SHG/TPEF images showing the changes over time at selected points across the tissue. Also of note is the increased TPEF signal at the top (fibrosa) layer of images over the degradation process, suggested to be cleaved elastin fragments floating upwards in the solution and possibly related to swelling

Microscopy was performed at the Neuroscience Center Microscopy Core Facility

nc state ENGINEERING