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Mesostructural changes of heart valve tissue during collagenase degradation

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Introduction and Background

Current Knowledge

- Valvular interstitial cells (VICs) catabolize damaged collagen fibers and help to repair tissues. Severe collagen depletion caused by matrix metalloproteinases (MMPs) induces tissue matrix destruction, altering the viscoelastic property of the heart valve tissues.
- Collagen degradation affects cellular regulations controlled by VICs, and can lead to heart valve diseases.

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
- An application of (0.5 mg/mL) collagenase for collagen degradation is used to simulate effects of MMPs
- Porcine aortic valve specimens are secured at a zero strain state and immersed in PBS or collagenase solution
- Multiphoton Second Harmonic Generation (SHG) imaging of collagen is performed during the degradation process at 30 min intervals for 180 min

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
- Skewness of the pixel intensity histogram is used as a depth independent measure of collagen concentration
- Fast Fourier Transform (FFT) is performed then power spectrum analysis to fit a gaussian model to the angular data to quantify organization

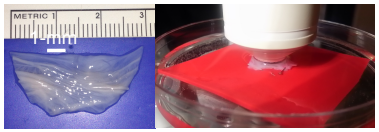

Fig. 1: Typical AV and experimental setup

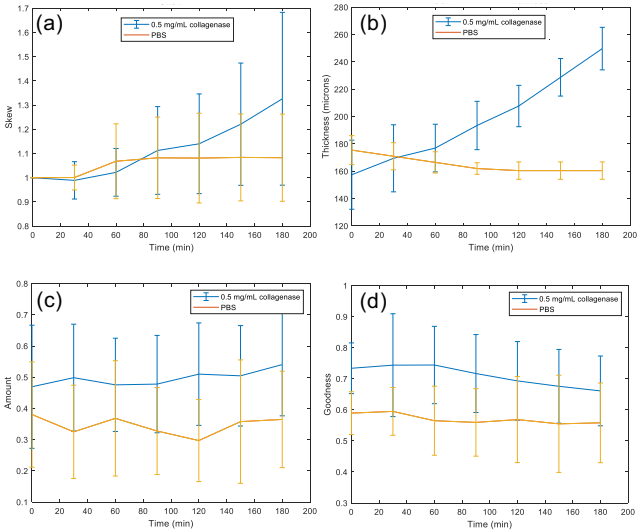

Fig. 2: Plots of (a) skewness of the pixel intensity histogram (b) thickness of the local collagen layer (c) amount of fibers contained within 1 deviation and (d) goodness of fit of the gaussian model of fiber organization

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, but variance remains high and no conclusions can be drawn from this as of now
- The degradation process allows a relaxation of the tissue causing it to swell and expand in overall thickness 59% on average
- Alignment and structure of collagen do not appear to show any significant changes over time based on FFT analysis, although the experimental group seems to have better fits than the control group the variance is high

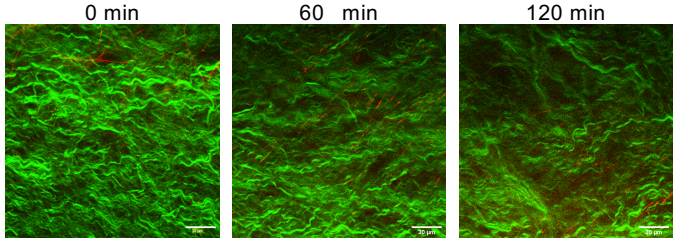

Fig. 3: SHG/TPEF images of collagenase tissue displaying collagen (green) and elastin (red) at time points of 0, 60 and 120 min at the centre of the collagen layer

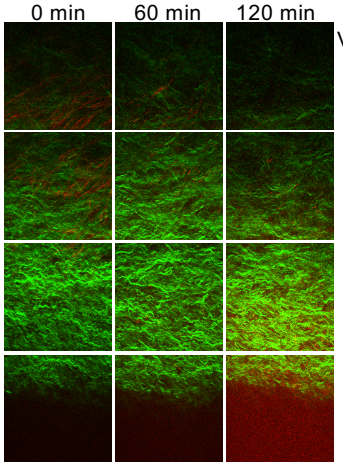

Fig. 4: MP SHG/TPEF images showing the changes over time at selected points across the tissue. Also of note is the increased TPEF signal at the top (fibrosa) layer of images over the degradation process, indicating potential for future analysis of the elastin component

Microscopy was performed at the Neuroscience Center Microscopy Core Facility

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Investigation, Modeling, and Reconstruction of the Tendon-to-Bone Insertion

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Introduction and Background

Current Knowledge

- Tendon-bone insertion tissue is structurally and functionally graded to alleviate stress concentration from soft tendon to hard bone
- Gradation in microstructure is not recreated in a healing insertion as in a native tissue

Current Limitations

- There is insufficient understanding of tissue microstructure and the property governing regeneration and repair post injury
- There is no comprehensive, three-dimensional mathematical model that may be used for modeling the insertion tissue

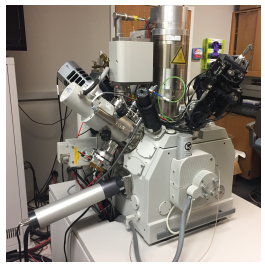
Objectives and Approaches

- Scans of tissue and density values at points within tissue obtained using micro-computed tomography
- Focused ion beam scanning electron microscope used to image microstructure of tissue at multiple depths throughout tissue
- Images obtained from scanning electron microscope pieced together in ImageJ to create 3D rendering of tissue

Methods and Results

Imaging and Scanning

- Imaging and scanning was performed using digital flexor tendon-bone units procured from the local abattoir (Nahunta Pork Center, Pikeville, NC) immediately after slaughtering.
- Tendon-to-bone connections were dissected from the two middle digits on the pigs' feet immediately after obtaining the feet. Samples were immediately fixated and critical point dried before imaging and scanning.
- Imaging was done using a focused ion beam and scanning electron microscope (FIB/SEM). A milling current and voltage of 7 nA and 30.0 kV, respectively, was used for milling, and the SEM beam voltage used was 5.00 kV (**Fig. 1**)
- Scanning was done using Micro-CT (Bruker SkyScan 1174, Billerica, MA). Scanning was done with a source voltage of 50 kV, source current of 755 µA, and image pixel size of 9.16 µm (**Figs. 2-3**).



FIB/SEM Specifications:

High Resolution SEM (Field Emission)
1nm resolution
Omniprobe Micromanipulator
FIB lift-out; in situ manipulation
Gas Injection System
Pt Deposition
Enhanced Carbon etch
Protochips *in-situ* Heating & Electrical Stage

Fig.1: FIB/SEM used for Scans

Fig. 2: Micro-CT Scanner

Micro-CT Specifications:
- 50kV Tungsten X-ray source
- 6µm 3D spatial resolution
- 2D/ 3D image analysis and realistic visualization

Fig. 3: Micro-CT Sample Mount

Results and Discussion

Density

- Density values at locations within cross-sections of the scanned tissue were obtained with DataViewer postprocessing software (Bruker, Billerica, MA).
- Density values, coupled with visual evidence, clearly indicate the location of the insertion, as well as provide a baseline for further analysis using FEM software (**Figs. 4-5**).

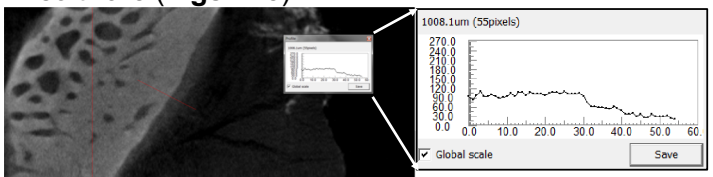


Fig. 5: Density Plot Along Insertion

Fig. 4: Cross-Section of Insertion Scan

Microstructure

- The microstructure of the tissue is seen via SEM images obtained using the FIB/SEM (**Fig. 6**).

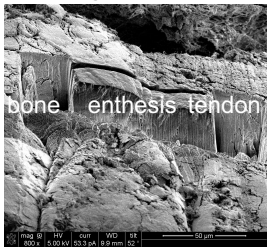


Fig. 6: FIB Milling Setup

Collagen fibers are clearly shown in **Figure 7**. Image reconstruction was performed in ImageJ (**Fig. 8**), with the end result being a 3D model that is to be import into ANSYS for further examination.

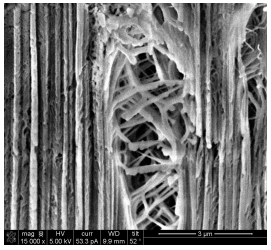


Fig. 7: Tissue Microstructure

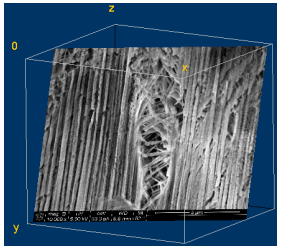


Fig. 8: 3D Image Reconstruction in ImageJ

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Orientation and Density of the Elastin Microstructure in the Jugular Venous Valve

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Introduction and Background

Motivation

- Incompetent venous valves lead to Chronic Venous Insufficiency(CVI), characterized by symptoms of swelling, ulcers, itching and pigmentation [3].

Current Limitations

- Information of tissue-level orientation of the elastin microstructure is currently unavailable, but hypothesized to play an important function in the overall mechanics and physiological function of the tissue.

Figure A, A Healthy Bi-Cuspid Valve

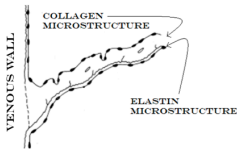


Figure B, Cuspid Anatomy

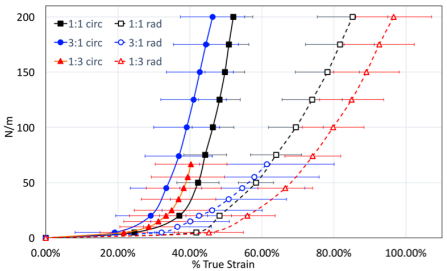


Figure C, Force-Control Biaxial Mechanical Testing

Summary of Approach

- The elastin microstructure of the bovine jugular venous valve is isolated, quantified, and imaged.
- The study is supplemental to the previously investigated force-control testing.

References:

[1] Cleveland Clinic, "Chronic Venous Insufficiency (CVI)."
[2] Tseng, H., and Grande-Allen, K. J., 2011, "Elastic fibers in the aortic valve spongiosa: A fresh perspective on its structure and role in overall tissue function," Acta Biomater, 7(5), pp. 2101-2108.
[3] R. Gottlob, R. May. 1986 "Venous Valves Morphology Function Radiology Surgery.".

Methods and Results

Isolation of Elastin Microstructure

- A time modified 75^{O}C 0.1 N NaOH treatment was used to digest all soluble collagen content [2].
- Time was increased in 15 minute intervals for NaOH treatments (n = 3 samples / time).

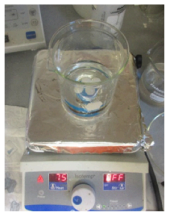


Figure D, 0.1N NaOH Treatment

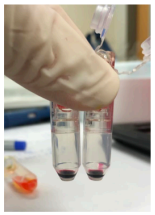


Figure E, Collagen Pellets after 30 minute Trial

- It was found that a 75 minute 75^{O}C 0.1N NaOH treatment was appropriate for digesting all collagen content and isolating the elastin microstructure.

Percent Dry Weight of the Elastin Microstructure

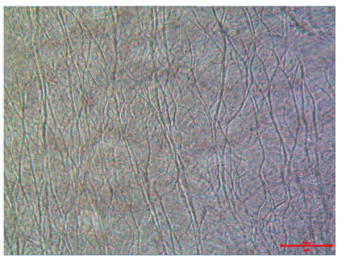
- Dry weights were attained via lyophilization before and after elastin isolation (n = 6).

Table A, Percent Dry Weight of the Elastin Microstructure

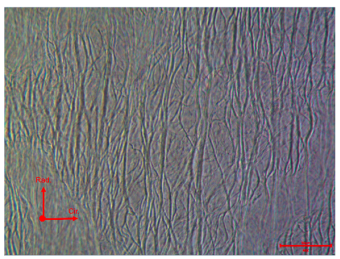
Sample	JV1	JV2	JV3	JV4	JV5	JV6
Percent Dry Weight of the Elastin Microstructure	9.84%	14.00%	12.50%	10.77%	7.84%	14.81%

- The elastin microstructure composes 11.63±2.64% dry weight of fresh valvular tissue.

Imaging the Elastin Lamina of the Venous Valve via Light Microscopy



Sample 1, Stitched Image of Isolated Elastin Microstructure



Sample 2, Isolated Elastin Microstructure

- Images focused in the belly region of the isolated wlastin microstructure.

Discussion and Conclusion

Elastin Microstructure During Mechanical Loading

- The elastin microstructure's crosslink mesh orients mostly radial, and the wavy parallel collagen fibers orient circumferentially.

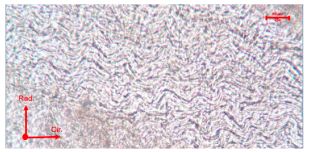


Figure F, Collagen Microstructure

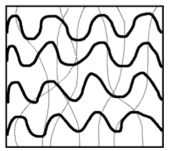


Figure G, Depiction of Collagen and Elastin

- The highly directional dependent microstructures result in anisotropic planar properties.

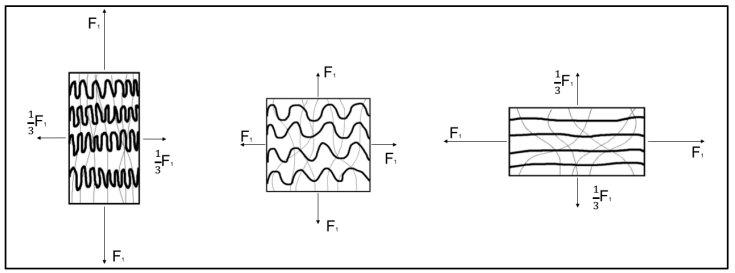


Figure H, Biaxial Loading Ratios

- During Different loading conditions (Figure H,C) the crosslinked elastin microstructure can rotate and support biaxial loads, but the collagen microstructure will remain circumferentially oriented.

Elastin Microstructure Physiological Function

- Physiologically the luminal side is advantageous for the elastin location because the luminal side experiences maximum stretch during anterograde flow.
- The elastin microstructure's tensile load is hypothesized in having the ability to close the valve during retrograde flow.

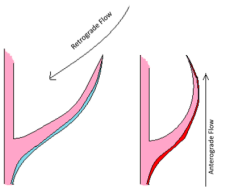


Figure I, Elastin Physiological Loading

- This physiological function emphasizes the importance of both elastin's density and it's orientation investigated in this study.

Conclusion

- To the best of the author's knowledge, the current study provides the first time isolation of elastin in the venous valve as well as an investigation of fiber rotation during loading.

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Constitutive Modelling of Jugular Vein Tissue

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Introduction and Background

Motivations :

- Highly Non Linear Stress - Strain behavior Mechanical Anisotropy, Large Deformations, Viscoelastic nature of the material.
- Veins (ubiquitous throughout the body) affected by numerous diseases like CVI, Thrombosis; used in valve replacement, vein reconstruction .etc., but without any proper study.
- Material Properties are not known.

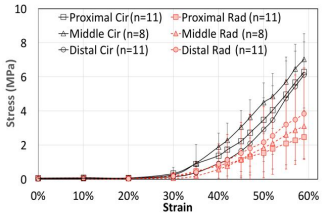

Fig. 1: General stress – strain behavior of jugular vein valve tissue showing high degree of anisotropy from a previous study. Results are averaged for number of samples due to high sample variability.

Focus :

I. To determine suitable phenomenological strain energy based constitutive relation for JV valve and JV wall tissue.

II. Determination of material parameters required to implement the model numerically in FEM environment.

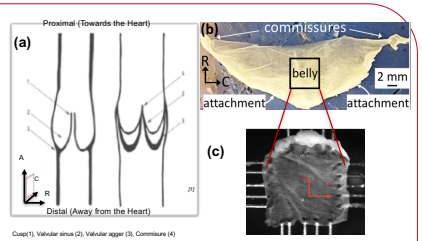

Fig. 2: Schematic showing the position of the tissue used for experimental tests: (a.) Position of the wall tissue. (b.) Position of the valve tissue sample from the belly region. (c.) Valve sample mounted on the biaxial testing apparatus with hooks attached for holding all 4 sides with no in plane shear.

Methods and Results

Constant Invariant Testing and Strain Energy based Constitutive Relation :

- Constant α and Constant I_1 Tests for 16 wall samples and 8 valve samples were conducted to study the behavior of strain energy derivatives.
- Response curves (Fig. 3) were plotted based on the experimental data.
- Separate strain energy descriptor were selected based upon the behavior.
- Wall Tissue:

$$W = c_1(\alpha-1)^2 + c_2(\alpha-1)^3 + c_3(I_1-3) + c_4(I_1-3)(\alpha-1) + c_5(I_1-3)^2$$

- Valve Tissue :

$$W = c_0(exp^{(c_1(I_1-3)^2 + c_2(\alpha-1)^4)} - 1)$$

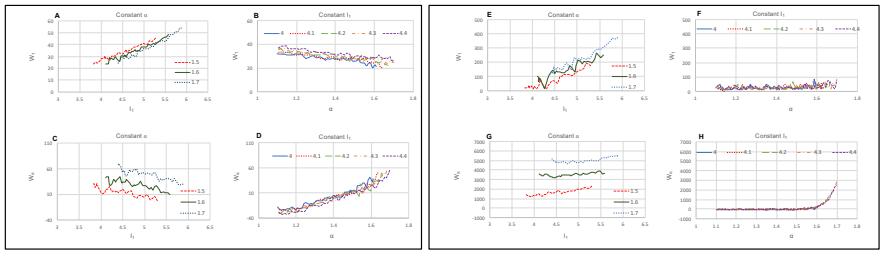

Fig. 3: Response curves generated from the constant invariant tests for wall (A-D) and valve (E-H) samples.

Material Model Parameter estimation :

- Using Powell's Method Algorithm to minimize the square of residuals between experimental and model predicted stress value. $\chi^2 = \sum_{i=1}^{N} [y^i - t^i]^2$
- Data from equibiaxial and off biaxial displacement controlled testing (with a maximum strain of 70%) was using for parameter estimation.
- 5 different protocols were used to collect data at 1Hz with 8 cycles of tissue preconditioning.

Sample	c_1	c_2	c_3	c_4	c_5
Proximal Wall Tissue	30.655	27.957	1.968	-17.916	5.205

Sample	c_0	c_1	c_2
Jugular Vein Valve Tissue	112.281	0.059	1.09

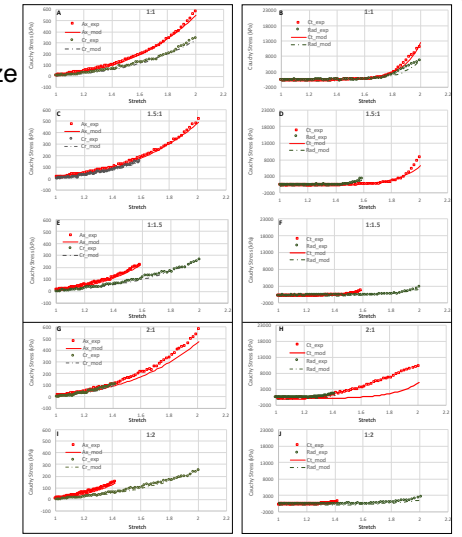

Fig. 4: Experimental vs Model Predicted Stress – Stretch data for wall (left panel) and valve (right panel) tissue for all 5 biaxial testing protocols.

Discussions and Conclusion

- Material models presented for the wall & valve tissue were able to emulate the behavior of tissue under experimental testing, evident from high correlation coefficients.
- The study also provides investigators with representative material parameters to form a continuum model when such is required for numerical analyses and computational simulations.
- Additionally, it can be of great help during the primary stages of bio prosthetic designs, valve-replacement surgeries, and when investigating valvular diseases.

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Mechanical Testing and the Cellular Microstructure of the Jugular Venous Valve Leaflet

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Introduction and Background

Current Knowledge

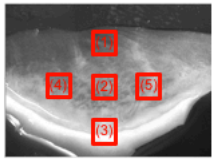
- Chronic Venous Insufficiency (CVI) occurs when the venous valves in the vein are damaged or malfunctioning leading to insufficient blood return to the heart and causes swelling in the legs.
- CVI effects up to 40% of the United States population, most frequently occurs above the age of 50, and is more common in women [1].

Current Limitations

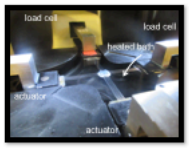
- Information of tissue-level mechanical property and cellular level microstructure of venous valve tissue is currently unavailable, hindering the development of bioprosthetic venous valve replacement.

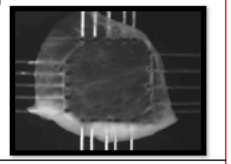
Summary of Approach

- As a model to move forward, the bovine jugular venous valve tissue is characterized by a Zeiss 710 Confocal Microscope to obtain deep tissue imaging information of elastin and fibroblasts in five regions.



Regions of Interest
(1) Free edge region
(2) Belly region
(3) Attachment region
(4) Left region
(5) Right region





References:

[1] Cleveland Clinic, "Chronic Venous Insufficiency (CVI)."
[2] Tseng, H., and Grande-Allen, K. J., 2011, "Elastic fibers in the aortic valve spongiosa: A fresh perspective on its structure and role in overall tissue function," Acta Biomater, 7(5), pp. 2101-2108.
[3] Neidlinger-Wilke, Grood, Claes, and Brand, 2002, "Fibroblast orientation to stretch begins within three hours," J Orthop Res, 20(5), pp. 953-956.
[4] Schleip, Findley, Chaitow, and Huijing, 2012 "Fascia: The Tensional Network of the Human Body: The Science and Clinical Applications in Manual and Movement Therapy." Edinburgh: Elsevier.

Methods and Results

Deep Tissue Imaging via Zeiss 710 Confocal Microscope Z-stack Technique

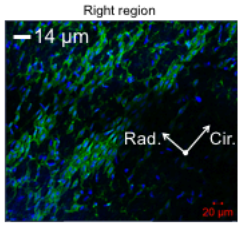


Figure A

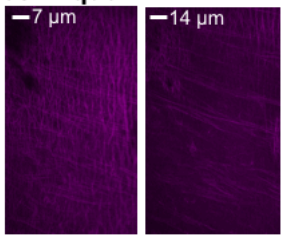


Figure B

- **Figure A** shows the right region's fibroblasts aligned in the circumferential direction stained by DAPI and Alexa Fluor 488 Phalloidin.
- **Figure B**, a picture from the belly region demonstrates that elastin changes with depth from being radially to circumferentially oriented on the inflow side of the valve.

Isolation of Elastin

- The valve tissue was treated with an adopted technique by soaking 0.1 N NaOH for 45 minutes at 75°C to isolate elastin [2].
- Images are taken from the attachment region.
- Verified 561 nm autofluorescence excitation was elastin fibers.

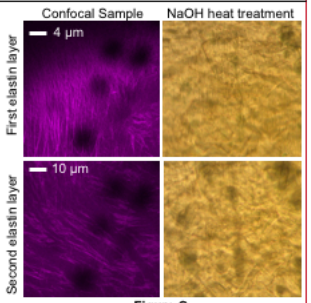


Figure C

Force-Control Biaxial Mechanical Testing

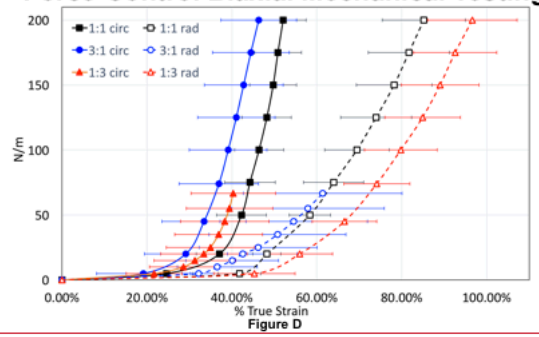


Figure D

Discussion and Conclusion

3-D Image of Cellular Microstructure and Elastin Fibers

- Fibroblast cells orient themselves in the circumferential direction of the venous valve leaflet in all regions of interest (**Figure E**). Fibroblasts cells elongated shape suggests they are active (**Figure A**).
- According to Neidlinger-Wilke fibroblast tend to orient themselves away from stretch [3]. Since, fibroblasts are oriented circumferentially it means the valve is stretched considerably more in the radial direction.

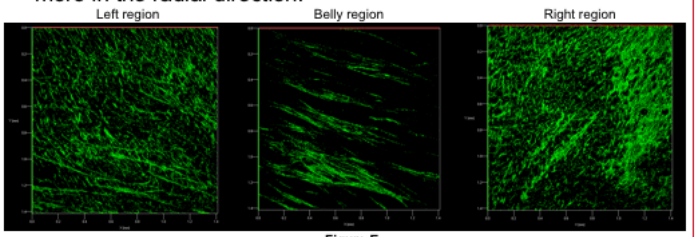


Figure E

- Another indicator of stretch predominantly in the radial direction is that most elastin is oriented radially (**Figure B**). Elastin is known to be the fiber that can stretch to 150% of it's original length [4]. So for mechanical purposes it makes sense that the elastin would align mostly in the radial direction where it's being stretched based on fibroblast orientation.
- Elastin diminishes form the attachment region to the free edge region (**Figure F**).

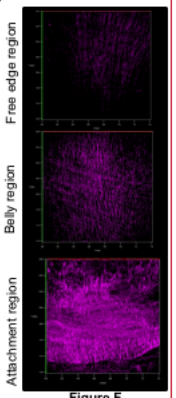


Figure F

Force-Control Biaxial Mechanical Testing

- Stress vs. strain curves have been exported from the data (**Figure D**) and can be used as part of a model in order to explain fiber orientation in the extracellular matrix during loading.
- The information will be used to construct an image-based finite element models to predict stress distributions in venous valves.

Conclusion

- To the best of the author's knowledge, the current study provides the first time of detailed 3D image of cellular level microstructure in venous valve tissues and force-control mechanical testing.

Acknowledgment: The study was supported in part by the Undergraduate Summer Research Grant from the Office of Undergraduate Research at NC State

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Biomechanical and Structural Properties at Tendon-Bone Insertion

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Introduction and Background

Current Knowledge

- Tendon-bone insertion tissue is structurally and functionally graded to alleviate stress concentration from soft tendon to hard bone
- Gradation in microstructure is not recreated in a healing insertion as in a native tissue

Current Limitations

- There is insufficient understanding of tissue microstructure and the property governing regeneration and repair post injury
- It is unclear how to reproduce the native structural inhomogeneity through implants and surgical reattachment

Objectives and Approaches

- Nonlinear and anisotropic *in situ* response of insertion tissue is examined via equilbiaxial testing using strain control protocol
- Strain rate dependent mechanical behavior is studied by generating biaxial tension data at varying strain rates
- Variation of tissue tangent moduli is observed at slow and fast strain rates across three different strain ranges
- Degree of anisotropy is examined by comparing stress-strain data along and transverse to the physiological loading direction
- Microstructural inhomogeneity is examined based on collagen and elastin structure variation across the width and depth of the insertion tissue through brightfield microscopy

Methods and Results

Mechanical Testing: Strain Rate Dependency

- Experiments were performed on porcine forelimbs (i.e., digital flexor tendon to coffin/pedal bone). Porcine trotters from large were obtained from the local abattoir immediately after slaughtering and were returned to the laboratory within 60 minutes of sacrifice for dissection.
- Equilbiaxial pre-load of 10mN was applied to release residual stresses inside tissue, followed by 11 preconditioning cycles of stretch up to 35% true strain and a 30s rest period.
- Porcine digital flexor tendon insertion specimens were immersed in HBSS at 37°C and stretched equilbiaxially on the BioTester 5000 (Fig. 1) at 7%/s and 15%/s to a peak of 75% true strains in two separate experiments.

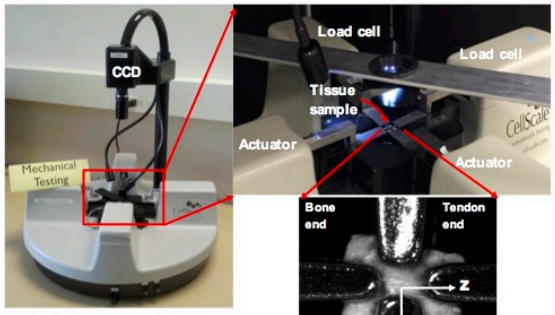


Fig. 1: BioTester for mechanical testing

x = medio-lateral
z = longitudinal
scale bar = 1 mm

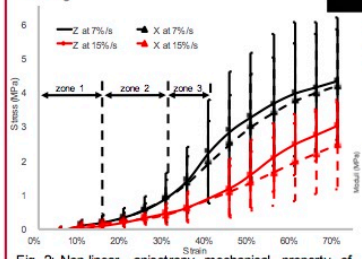


Fig. 2: Non-linear anisotropy mechanical property of tendon-bone insertion tissues at different strain-rates

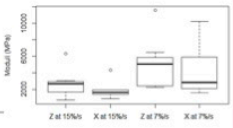


Fig. 3: Comparisons of moduli at zone 3

Results and Discussion

Histology

- Histological slides (with Hematoxylin & Eosin (H&E) and Verhoeff-Van Gieson (VVG) stains) of the insertion in the sagittal (i.e., y-z plane in red) and horizontal (i.e., x-z plane in blue) planes are observed for through the Zeiss Axiophot microscope.

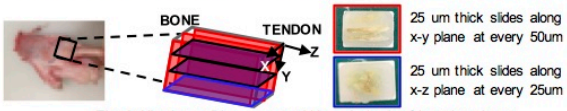
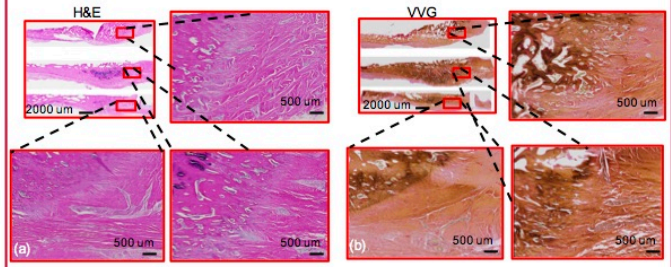


Fig. 4. Histology slides across width and depth of insertion tissue



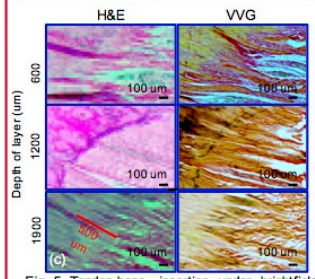


Fig. 5. Tendon-bone insertion under brightfield microscopy (water immersion, x400)

Transition in Microstructures

Collagen fiber orientation is largely along the physiological loading direction with no drastic reorientation across the width or thickness. However, fiber density is highest at mid-height (belly region) plane section

Mechanical Property of Insertions

In the equilbiaxial testing, increased stiffening beyond toe region is observed at the slower strain rate. Although the stresses along the tendon-longitudinal axis (z in Fig.2) are consistently higher, observed anisotropy is not significant.

Acknowledgment: We would like to acknowledge the funding by National Science foundation, under award number BMMB-1400018.

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Biaxial Mechanical Properties of Venous Valve Leaflet Tissues

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Introduction and Background

Current Knowledge

- Incompetence of venous valves plays an important role in chronic venous insufficiency.
- Valvular tissues have stronger mechanical properties than vein wall tissues under uniaxial tensile testing [1].

Current Limitations

- The native loading conditions of venous valves should be multi-axial. However, only uniaxial tensile testing has been reported [1].
- Quantitative information of extracellular matrix components of the valve is not available.

Objectives

- To obtain stress-strain curves and modulus of elasticity of venous valve tissues under biaxial tensile testing.
- Quantitatively analyze collagen concentration of venous valve tissues.

The results will provide information for future tissue engineered venous valve designs, with matching mechanical properties and biochemical components of native venous valve tissues.

Methods

Equibiaxial Tensile Testing

- Bovine jugular venous valve (JV) and saphenous venous valve (SV) specimens were collected.

Collagen Assay

- The specimens were mixed with collagen extraction solution for 120 hours. Collagen concentration of each specimen were analyzed afterwards.

Results

Bovine JV and SV Samples Preparation

- JV (10mm x 10mm) and SV (2mm x 2mm) specimens were immersed in HBSS at 37°C and stretched by a biaxial tester to 60% in both circumferential (cir) and radial (rad) directions.

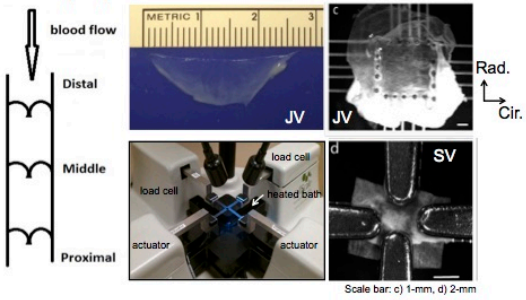


Fig.1 JV stress-strain curves

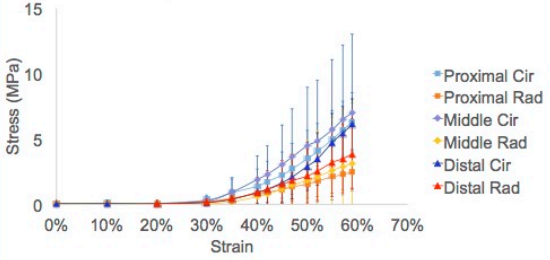
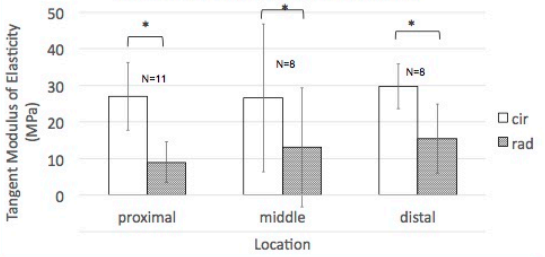


Fig.2 Inter-valvular modulus variability

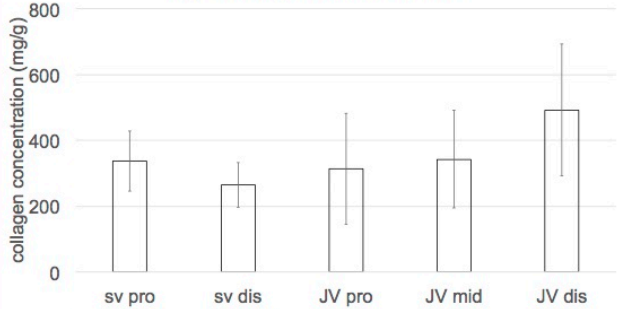


Discussion and Conclusion

Bovine JV and SV Mechanical Properties and Collagen Concentration

- Venous valve leaflet tissues showed anisotropic and nonlinear mechanical properties (Fig.1).
- Tangent moduli of elasticity in the Cir direction of the valve were larger than those in the Rad direction, indicating the leaflet tissues were stiffer in the Cir direction (Fig. 2).
- JV tissues showed an increasing trend in collagen concentration from the proximal end to the distal end, while SV tissues showed a decreasing trend (Fig. 3).

Fig. 3 Collagen concentration



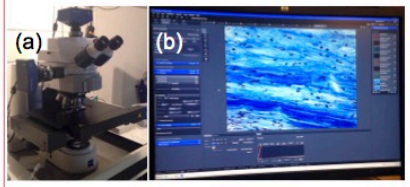


Fig. 4. State-of-art Zeiss Axiophot microscope in the Cellular and Molecular Imaging Facility at NC State.

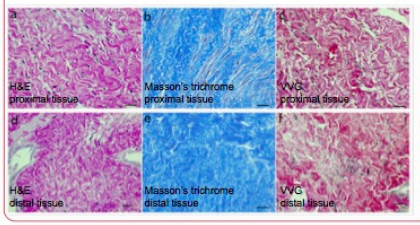


Fig. 5. Histological images of JV tissues under 400x with H&E, Masson's trichrome and VVG stains, (a-c) Proximal tissue (d-f) Distal tissue, scale bars: 50um

[1] J. S. Ackroyd, M. Pattison, and N. L. Browse, *Br. J. Surg.*, vol. 72, no. 2, pp. 117–119, 1985.

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The Stress-Relaxation Behaviors of Diseased Heart Valve Tissues

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Introduction and Background

Current Knowledge

- Valvular interstitial cells (VICs) catabolize damaged collagen fibers and help to repair tissues. Severe collagen depletion caused by matrix metalloproteinases (MMPs) induces tissue matrix destruction, altering the viscoelastic property of the heart valve tissues.
- Collagen degradation affects cellular regulations controlled by VICs, and can lead to heart valve diseases.

Current Limitations

- It is unknown how collagen fibers are selectively catabolized or how MMPs differentiate damaged collagen fibers from functional collagen fibers.

Objectives and Approaches

- An approach to understand if **strain level** plays a role in the **selective degradation** of collagen fibers is performed via the testing of stress relaxation.
  - * An application of collagenase for collagen degradation is used to simulate effects of MMPs.
  - * A series of stress relaxation tests are conducted under different strain levels and collagenase concentrations:

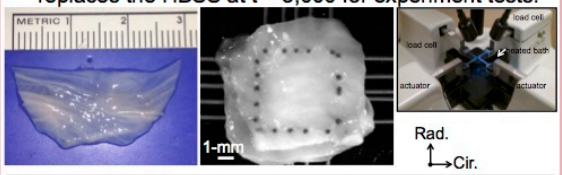
Strain (Cir.)	Strain (Rad.)	Collagen Conc.
20% (AV)	70% (AV)	0.2 mg/ml
20% (PV)	90% (PV)	0.5 mg/ml

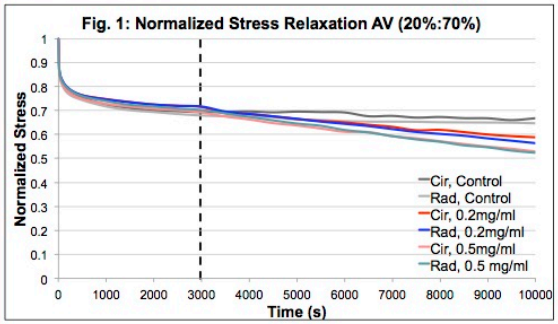
AV: Aortic Valve; PV: Pulmonary Valve
Cir.: Circumferential; Rad.: Radial

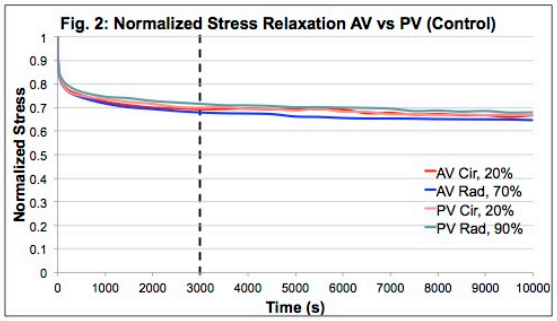
Methods and Results

Stress Relaxation under Stretching and Collagen degradation

- Porcine AV and PV specimens (10mm X 10mm) are immersed in HBSS at 37°C and stretched by a biaxial tester under different strain levels.
- Two specimens are held at the assigned strain level for 10,000 seconds (about 3 hours). Collagenase replaces the HBSS at t = 3,000 for experiment tests.







Discussion and Conclusion

Influence of Collagen Degradation on Mechanical Properties of Heart Valve Tissues

- The normalized stress in AV decreases as collagenase concentration increases, indicating a degradation of collagen fibers with increased simulation of MMPs (Fig.1).
- Valves tested at the physiologically accurate strain levels results in equal stress for both AV and PV (Fig. 2).
- Based on previous results [1], stretching valves at strain levels physiologically accurate for a normal heart (rather than equi-biaxial) may strengthen collagen fibers, aiding in resisting degradation from MMPs (Fig. 3).

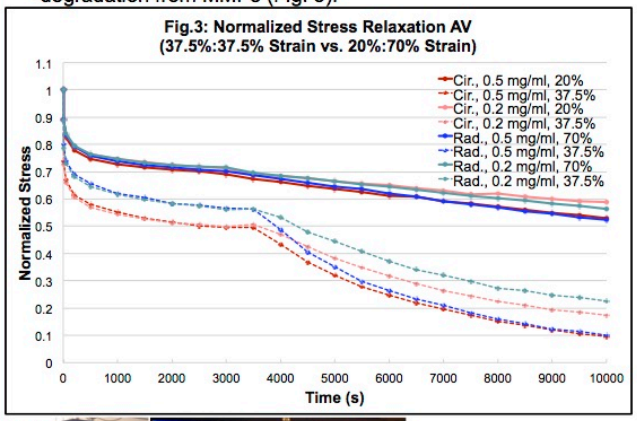


Fig. 4. State-of-art Zeiss Axiophot microscope in the Cellular and Molecular Imaging Facility at NC State.

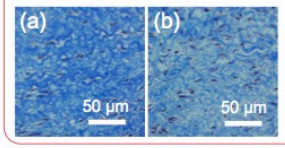


Fig. 5. Histological images of AV tissues (with Masson's trichrome stain), 400x, tested at **(a)** 0.2 mg/ml and **(b)** 0.5 mg/ml collagenase concentrations.

[1] Huang, S., & Huang, H. S. (2015). *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine, 229*(10), 721-731.

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Examination of Biaxial Mechanical Properties of Tendon-Bone Insertion

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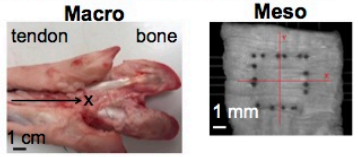
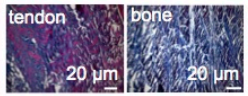
Introduction and Background

Current Knowledge

- High strain-rate stretching along the direction of fibers can damage the tendon-bone insertion irreversibly.





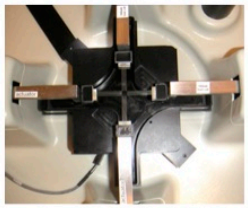
***Micro**-structure of collagen fibers are related to the mechanical property*

Current Limitations

- How stretching the tendon-bone insertion biaxially affects its properties is currently unknown.

Objectives and Approaches

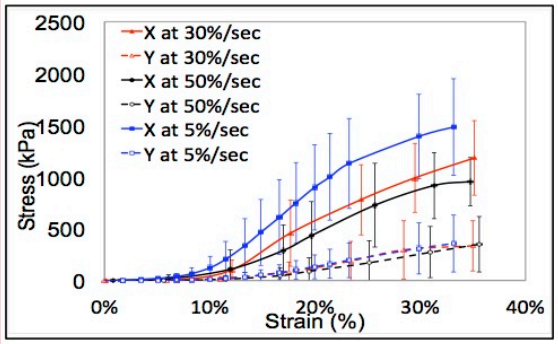
- Properties of longitudinal cross section tissue samples will be tested via a Biotester 5000 (CellScale, Waterloo, CAN).
  - * Design a mounting system for the machine to test the stiffer tendon-bone insertion sections
  - * Draw comparisons between mid tendon and tendon-bone insertion properties.

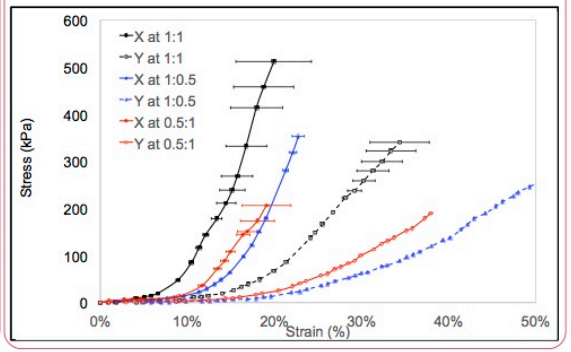


Methods and Results

Biaxial Stretching using Displacement and Force Control

- Porcine tendon specimens (5mm X 5mm) are immersed in HBSS at 37°C and biaxially stretched by a biaxial tester under different strain levels. (X: Longitudinal; Y: Circumferential).
- Specimens undergo 12 cycles of prestreching at 2%/sec strain.
- Mid tendon results suggest biaxial stretching can help protect the tendon from breaking

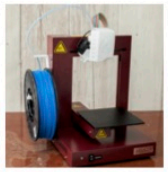


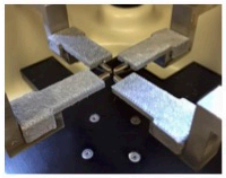


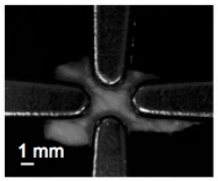
Gripping Design and Fabrication

Clamp Design and Ongoing Results

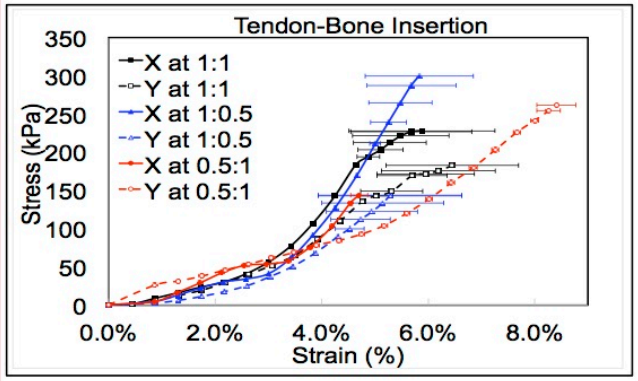
- A mount for the clamp was designed and 3D printed using an ABS polymer material.
  - * Alligator clips are used to hold the 2 mm X 2mm tendon-bone insertion tissue.
    - Clamps grip onto the tissue and stretch without slip.







- Relationships between the longitudinal and radial direction are discovered:
  - * Stress in the longitudinal direction is greater than the circumferential direction
  - * Stretching biaxially protects the tissue from breaking
  - * The study is ongoing and the mid tendon results will be compared to the tendon-bone insertion data



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The Stress-Relaxation Behaviors of Collagen-Depleted Heart Valve Tissues

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

Introduction and Background

Current Knowledge

- Severe collagen depletion caused by matrix metalloproteinases (MMPs) pathologically induces matrix destruction, changed viscoelastic property of the heart valve.
- Collagen degradation further affects cellular regulations mediated by heart valve cells, and even leads to heart valve diseases.

Current Limitations

- How viscoelastic properties of valve leaflet tissues may change during physiological or pathological remodeling is unknown.

Objectives and Approaches

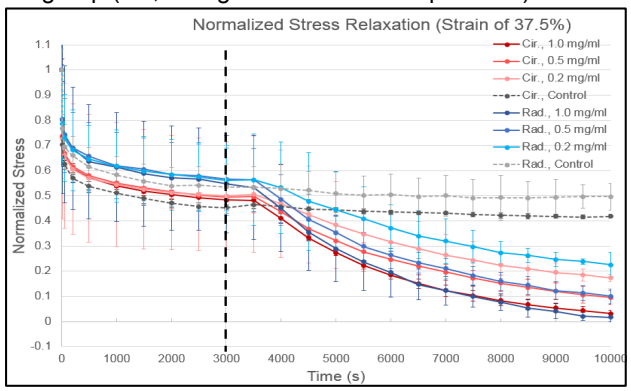
- An easy approach for the ***collagen-deficient heart valve tissue*** responding to ***the mechanical environment*** is performed via the testing of stress relaxation.
  - * An application of collagenase for collagen degradation is used to simulate effects of MMPs.
  - * A series of stress relaxation testing are conducted under different strain levels and collagenase concentrations:

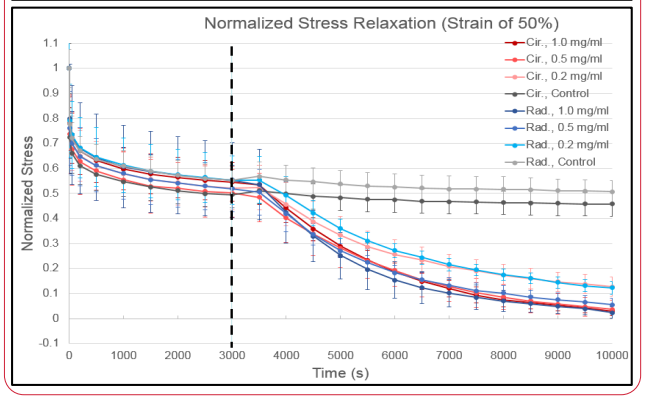
Strain	Collagen Concentration
37.5%	0.2 mg/ml
50%	0.5 mg/ml
	1.0 mg/ml

Methods and Results

Stress Relaxation under Stretching and Collagen degradation

- Porcine aortic valve (AV) specimens (10mm X 10mm) are immersed in HBSS at 37ºC and equi-biaxially stretched by a biaxial tester under different strain levels. (Cir.: Circumferential; Rad.: Radial).
- Specimens are hold at the assigned strain level in 10,000 seconds (about 3 hours). Collagenase is added at t = 3,000.
- Stress apparently drops in each condition after adding collagenase compared to the stress in the control group (i.e., collagenase-untreated specimen).

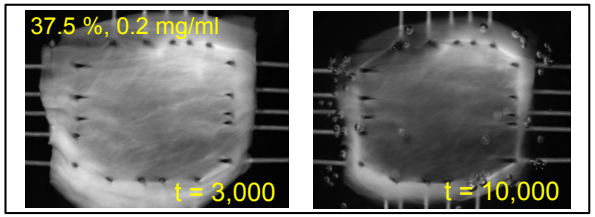




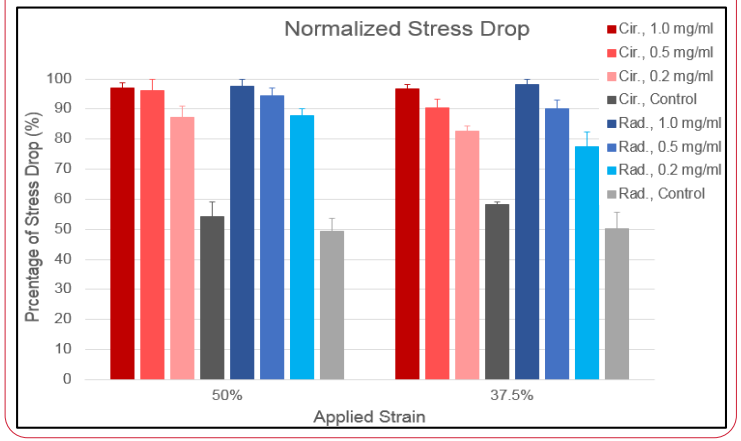
Discussion and Conclusion

Influence of Collagen Degradation on Mechanical Properties of Heart Valve Tissues

- From images of the specimen during stretching, the degree of ***transparency*** is different between the initial stage and the final stage during stress relaxation. ***Collagenase digests collagen*** and its concentration affect the degree of transparency of the tissue.



- Dependencies of ***fiber orientation***, ***stretching***, and ***collagenase concentration*** are discovered:
  - * Normalized stress relaxation in the circumferential direction is greater that in the radial direction.
  - * With larger strain levels, larger normalized stress drops are observed.
  - * Normalized stress relaxation is increased with collagenase concentration.



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Biomechanical Properties of Skin

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

Introduction and Background

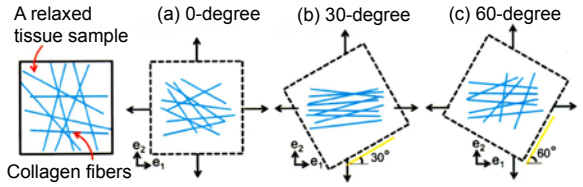
Current Knowledge:
Collagen fibers arrangements within skin tissue determine its strength and its anisotropic behavior.

Current Limitations:
How subjecting skin to stretching changes its biomechanical properties is unknown. Subjecting skin to stretching could reduce the anisotropic properties of skin, creating uniform material properties benefiting many medical applications.

Objectives: Determining the directional mechanical characteristics of skin tissue is key to understanding how the collagen arrangement affects the mechanical properties of skin tissue.

- To investigate the relationship between collagen fiber orientation and tissue strength via biaxial testing.
- To understand why subjecting skin to stretching has directional mechanical properties. Primarily, the difference in anisotropic properties are determined by comparing non-stretched and stretched samples.

- Collagen fibers are distributed randomly within skin tissue.
- When stretched, fibers align in a preferred direction, which causes the anisotropic properties.

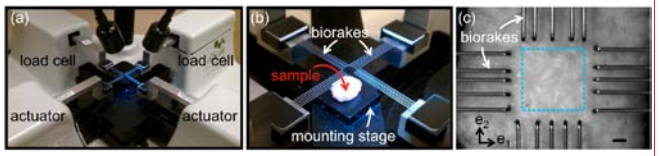


Methods and Results

Directional Mechanical Properties via Biaxial Testing

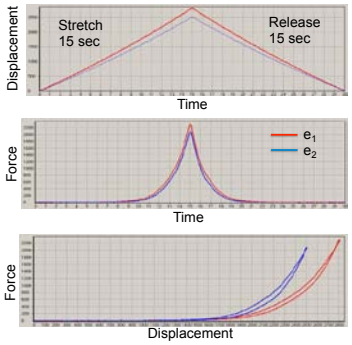
Equipment:

- Biaxial testing was conducted via a BioTester 5000.
- Porcine epidermis 4 mm square skin samples were tested bi-axially.



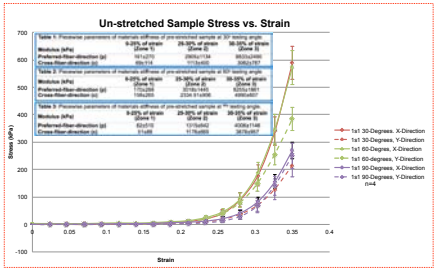
Procedure:

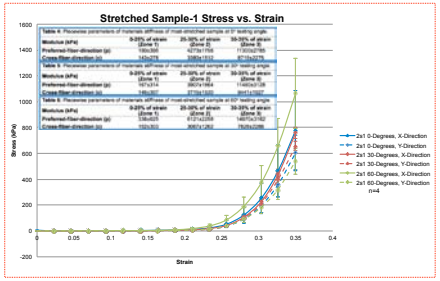
- Skin samples were stretched from 0% strain to 35% to 0% over 30 second time period.
- 3 different skin samples were considered:
  - Un-stretched
  - Stretched 1, experienced the most stretch
  - Stretched 2, experienced less stretch
- Stretched samples were subjected to stretching for 24hrs in balloon tissue expander.
- Each sample was tested five times at 0 degrees, 30 degrees and 60 degrees. Only tests 2-5 were considered.

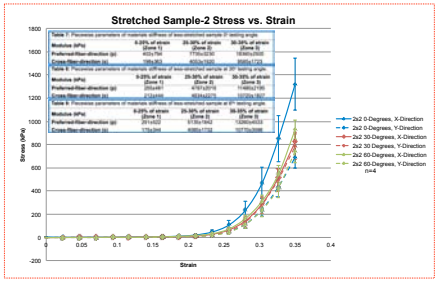


Directional Mechanical Properties in Skin Tissues

- Corresponding stress and material stiffness was calculated for each samples' rotation.
- Samples' mechanical properties become most anisotropic and non-linear in Zone 3.
- Comparing material stiffness of the preferred and cross-fiber directions in Zone 3 indicate reduced anisotropic properties.







Discussions and Conclusion

- Reducing anisotropic properties, which eliminates weaknesses, occurs after skin tissue experiences an expansion processes. The expanded skin better serves patients in need of skin graft operations. The current study provides new methods for determining and comparing the directional mechanical properties of skin tissue.
- The collagen fiber distribution in skin tissue becomes more homogeneous by subjecting samples to stretching, indicated by the similar material strengths in the X and Y-directions from the results of biaxial testing; however, preferred collagen alignment directions still exist.
- Longer periods of stretching may have a more significant affect on the reduction in anisotropic properties.

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A Synergistic Approach to Visualize and Understand Tissue-Cell Mechanical Interactions

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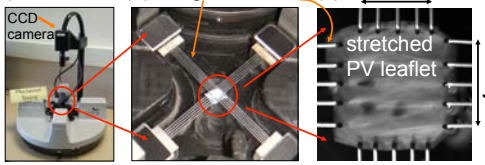
Introduction and Background

Current Knowledge:
Heart valves constantly experience different stress states during cardiac cycles.
Current Limitations:
How tissue-level mechanical forces can translate into altered cellular stress states is unknown, which hinders a better understanding of the relationships between the mechanical stimuli, cellular mechanotransduction, cell migration, matrix synthesis, and tissue remodeling.

Objectives: Capturing the true micro-structure of collagen fibers is key to understand how forces are being transferred to the cellular-level.

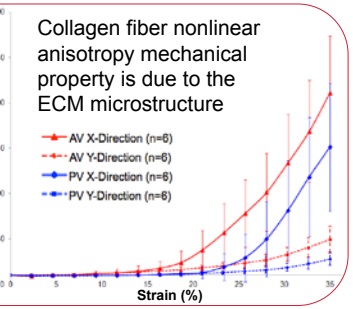
- To investigate stress distributions in heart valve tissues via an automated finite element analysis (FEA).
- To establish the relations between stresses around cells and measured cell nuclei deformation for better understanding cell mechanobiology.

(a) BioTester (b) Tungsten rakes (c)



- Biaxial testing was conducted via a BioTester 5000 (CellScale, Waterloo, CAN).
- Porcine pulmonary valve (PV) Leaflet samples were tested circumferentially (x-axis) and radially (y-axis).

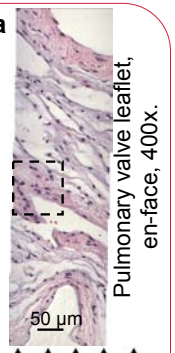
Collagen fiber nonlinear anisotropy mechanical property is due to the ECM microstructure

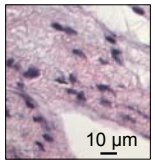


Methods and Results

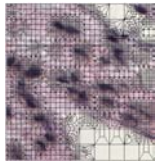
Microstructural Evolutions via Finite Element Simulations

- Histology were prepared with H&E stains. Photomicrographs were obtained via an optical microscope.
- Circumferential aligned collagen fibers were notable in pulmonary valve leaflets.
- The non-linear anisotropic, and heterogeneous nature of heart valve extracellular matrix is incorporated into our FEA.

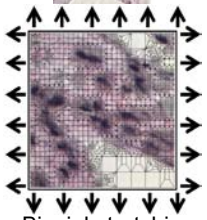




Histological photo-micrographs



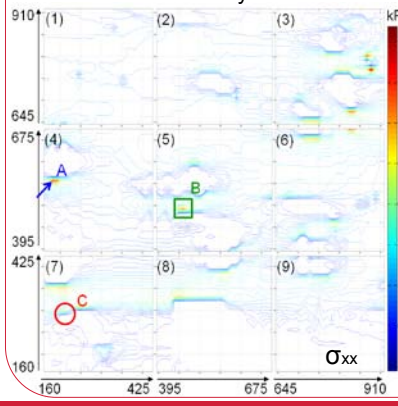
FEA mesh and skeleton

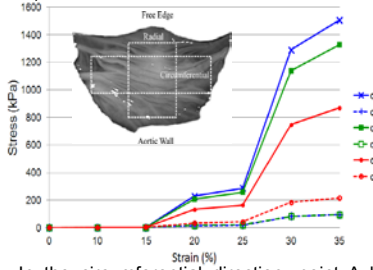


Biaxial stretching simulation

Stress Distributions in Heart Valve Tissues

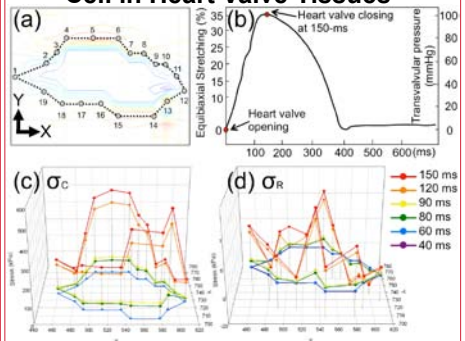
- The corresponding mechanical stress distributions under biaxial stretching around cells are computationally calculated via FEA.
- The feature of inhomogeneous microstructure is apparently illustrated, and most local peak stresses are located around nuclei.
- Higher mechanical stimulation occurs around the boundary, rather than in the belly area of the heart valve; therefore, cellular activities could be stronger around the boundary.





- In the circumferential direction, point A has a higher stress value than B because the strain energy on the boundary of tissue is higher than the middle.
- In the radial direction, C is closer to the boundary, so that C receives higher energy, hence it has a higher peak stress, comparing to point A.

Stress Evolutions around a Cell in Heart Valve Tissues



- The tissue sample is divided into 9 overlapping regions. The cell is selected in the region 2 with the defined boundary.
- σ_c is higher than σ_R, suggesting higher cellular activity may occur along the preferred fiber direction.

Discussions and Conclusion

- Cellular mechanotransduction might be directional-dependent due to higher stresses are observed in the circumferential direction via our finite element analyses.
- Current study provides new models for better understanding tissue-cell interactions in heart valves.
- Stress distribution is highly emphasized as a result of biomechanics related to collagen secretion, tissue structure integrity and remodeling influenced by mechanical forces.
- Virtual experiments of heart valve tissues provide translational models to clarify the force transmission roles of heterogeneously distributed collagen fibers and the force receiving roles of randomly distributed cells in the matrix.

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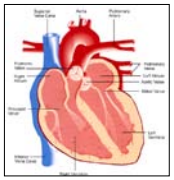
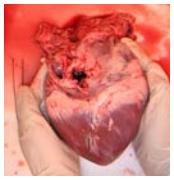
Multiscale Interactions of Mechanics, Microstructures, and Composition of Heart Valve Tissues

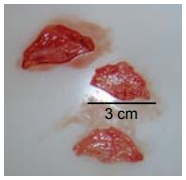
Brittany N. Balhouse[1], Ashutosh Garg[2] and Hsiao-Ying Shadow Huang[2,3]
[1]Biomedical Engineering, [2]Aerospace Engineering, [3]Mechanical Engineering, North Carolina State University, Raleigh, NC

Introduction and Background

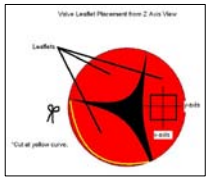
- Approximately 250,000 heart valve disease in USA in 2010[1]. Stenosis and insufficiency are the most common heart valve diseases, which are related to the mechanics of heart valves.
- Objective: Investigated the relationship of mechanical property, collagen fibers microstructure, and collagen concentrations in aortic and pulmonary semilunar valves.


Anatomy of the heart[4]


Porcine heart



Pulmonary valve leaflets

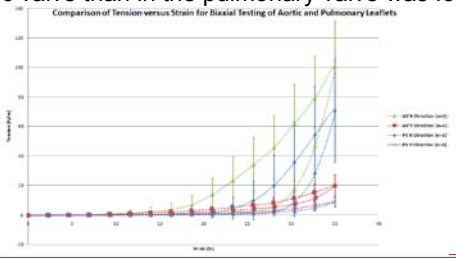

Method of dissection

- Function of heart valves:
  1. allow blood to flow through the heart smoothly.
  2. prevent retrograde flow of blood.
- Method of dissection:
  1. Each valve has three leaflets. Cartesian coordinate system was set before dissection.
  2. Leaflet samples were relaxed in HBSS for the physiological condition.

Method and Results

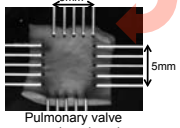
Mechanical Testing

- Biaxial testing was conducted via a BioTester 5000 (CellScale, Waterloo, CAN).
- ~7mm x 7mm sample were cut from leaflets.
- Samples were mounted and tested circumferentially (x-axis) and radially (y-axis).
- Evenly distributed boundary conditions were provided, which eliminates the variability between sample sizes.
- Aortic valves are stiffer in the circumferential direction.
- A greater variance in the directional strength of the aortic valve than in the pulmonary valve was found.

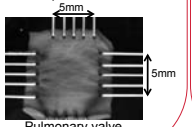



BioTester 5000


Tungsten BioRakes



Pulmonary valve sample, relaxed

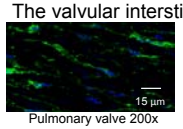


Pulmonary valve sample, stretched

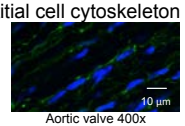
Cellular Analysis

- Tissue-Level:
  - Histology were prepared with Masson or H&E stains. Photomicrographs were obtained via Leica DMLB Microscope.
  - Circumferential aligned collagen fibers were notable in aortic valve leaflets.
- Cellular-Level:
  - Immunohistochemical samples were prepared for a confocal microscope.
  - Direction of actin filaments follows the nuclei long-axis.

The valvular interstitial cell cytoskeleton



Pulmonary valve 200x

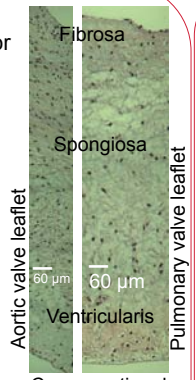


Aortic valve 400x

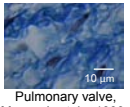
The valvular interstitial cell cytoskeleton. Actin filaments are shown in green and the cell nuclei are in blue.


Aortic valve, en-face, 400x


Pulmonary valve, en-face, 400x



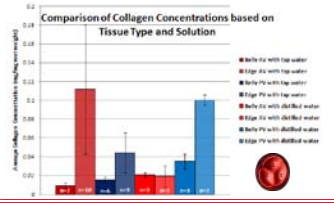
Cross-sectional view 400x



Pulmonary valve, Masson's stain, 1000x

Biochemical Analysis

- Collagen extracted from a leaflet sample into solution (0.5M acetic acid: distilled water = 0.029:1 & 50mg Pepsin A), dyed, centrifuged, and dissolved in Alkali reagent.
- A spectrophotometer and standard curve were used to calculate the concentrations.
- Use of distilled water and collagen extraction time may have substantial effects on results
- Higher collagen concentrations were observed on edge regions than on belly regions of PV leaflets (AV leaflets provided inconsistent location-dependent results)



Discussion

- A relationship exists between the mechanical strength, collagen fiber microstructure, and collagen concentration of a valve leaflet.
- Semilunar valve tissues have nonlinear anisotropy material properties due to the heterogeneous collagen fiber microstructure.
- A higher collagen concentration may be related to greater mechanical strength and may be location dependent.
- The mechanical property of semilunar valve tissues do not depend only on collagen concentration but how collagen fibers are arranged structurally at the microscopy level.

[1] American Heart Association.
[2] Courtesy of Chung, M.K., and Rich, M.W. *Alcohol Health and Research World 14(4):269–276, 1990.*

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Virtual Experiments of Extracellular Matrix-Cell Interactions of Heart Valve Tissues

Cory Burgett[1], and Hsiao-Ying Shadow Huang[2]
[1]Computer Science, [2]Mechanical and Aerospace Engineering, North Carolina State University, Raleigh, NC

Introduction and Background

- Objective: Develop an automated finite element analysis to study extracellular matrix-cell interactions in heart valves.
- The interactive content is made possible through the use of BioTester (Figure 1), photomicrographs (Figure 2), and an open source finite element software-OOF2, developed by National Institute of Standards and Technology[1].
- OOF2 wasn't designed to handle heart valve tissue unique microstructures

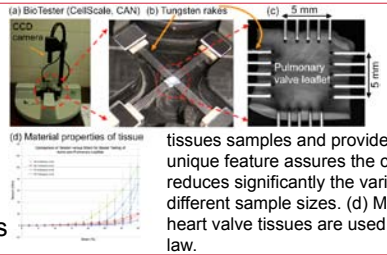


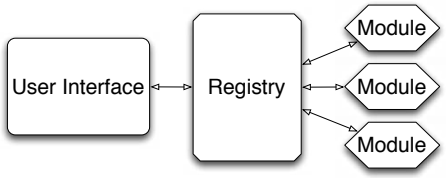
Figure 1: (a) The BioTester is capable of applying physiologically plausible biaxial loading states on tissue samples. (b)-(c) The tungsten rakes pierce through heart valve tissues samples and provide evenly distributed loading. This unique feature assures the control of loading conditions and reduces significantly the variability associated with testing different sample sizes. (d) Measured material properties of heart valve tissues are used to develop a matrix-constitutive law.

Figure 2, The microstructure of pulmonary valve tissue. Collagen fibers and extracellular matrix are shown in pink and the cell nuclei are in black (400x).

- Extension OOF2 for automated analysis of heart valve tissue is required.

Design and Implementation

OOF2 Architecture



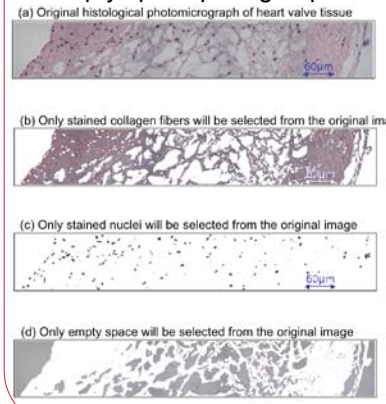
- Framework provides the UI and a Registry and is written in Python.
- Modules provide each individual feature. Some are written in Python while others are written in C++.
- Loaded modules are accounted for in the Registry. The UI uses registry information to pass user input to the modules for processing.
- The modules compute some output and pass it back to the user interface.

Extensions:

- Extensions were developed to better differentiate and describe photomicrographs of cells and collagen fibers in heart valve tissues.

1. Pixel Selection Extension

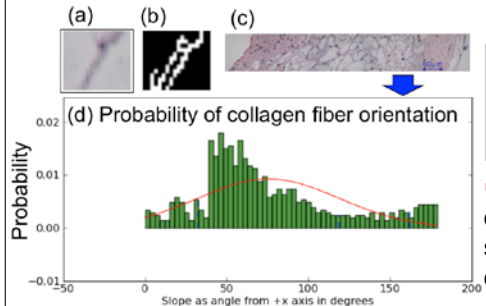
- Objective: Use image properties to differentiate between the collagen, nuclei, and space.
- Method: Based on hue, saturation, intensity color space from (a) an original image, the developed pixel selection extension are able to filter (b) only stained collagen fibers to a collagen fiber pixel group, (c) only stained nuclei to a nuclei pixel group, and (d) only empty space to an empty space pixel group.



- Outcome: Quickly assign material properties to all pixels in the image for more accurate simulation.

2. Materials Property Extension

- Objective: User-defined stiffness matrices for collagen fibers in the radial and the circumferential directions.



- Method: Hough Transform detects the slope of the strongest line displayed in each element.
- It is observed that most of the collagen fibers in are alignment with ~55 degrees from +x axis.
- The slope is used to calculate how much of each stiffness matrix contributes to the net stiffness for that element.

Discussion

- The automated finite element model captures heterogeneous cell and collagen fiber microstructures from heart valve tissue histological photomicrographs.
- Finite element analysis is performed on an image to conduct virtual experiments.

[1] OOF: Finite Element Analysis of Microstructures, Applied and Computational Mathematics Division National Institute of Standard and Technology, U.S. Department of Commerce.

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