# THE STRUCTURE, ORIENTATION AND DISTRIBUTION OF ELASTIC MATERIALS AND THEIR INFLUENCE ON THE VASCULAR WALL DYNAMICS OF HEALTHY ARTERIES


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## Background:

*Pulmonary Hypertension*

- Leads to vascular remodeling in the chronic situation. This structural change increases both the flow resistance of the distal arteries and the flow impedance of the proximal arteries.
- The increased afterload imposed on the heart leads to cardiac remodeling and eventually right ventricular failure.

*Arterial Mechanics and the Strain Energy Function (SEF)*

1. Exponential-Polynomial Strain Energy Function
   - Developed by Chuong and Fung (1983)
   - Phenomenological, assumes that arteries are composed of an isotropic material and have a uniform, thick-walled, cylindrical shape
   - Accurately describes the stress-strain behavior of arteries within physiological pressure limits.
   - Only moderately useful for the extraction of meaningful histological data regarding vascular remodeling.

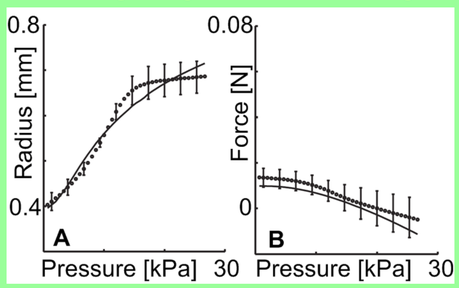



Fig. 1. Comparison of outer radius and axial force (dotted lines )with SEF developed by Choung and Fung (solid line). Zulliger M.A., Journal of Biomechanics 2004 (37) pp. 996

2. Semi-Structural Model
   - Developed by Holzapfel and Gasser (2000)
   - Introduces anisotropy by modeling elastin as an isotropic thick-walled cylinder helically wrapped by collagen at a characteristic angle.
   - More accurately describes the stress-strain behavior of the artery outside of physiological pressure ranges.
   - Is used to develop an understanding of the impact of collagen on arterial mechanics

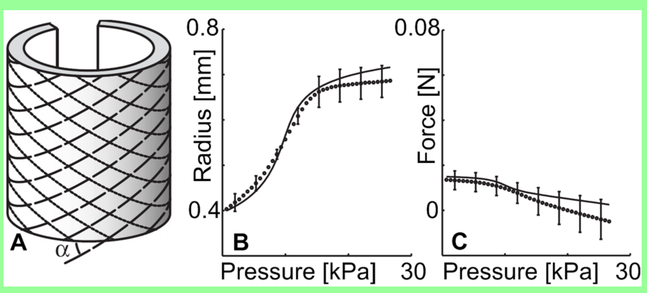



Fig. 2. Comparison of outer radius and axial force (dotted lines )with SEF developed by Holzapfel and Gasser (solid line). Zulliger M.A., Journal of Biomechanics 2004 (37) pp. 990 & 996

3. Refinements of the Semi-Structural Model
   - Zulliger and Fridez included the wavy configuration of collagen in the zero-stress state and the volume fractions of collagen and elastin

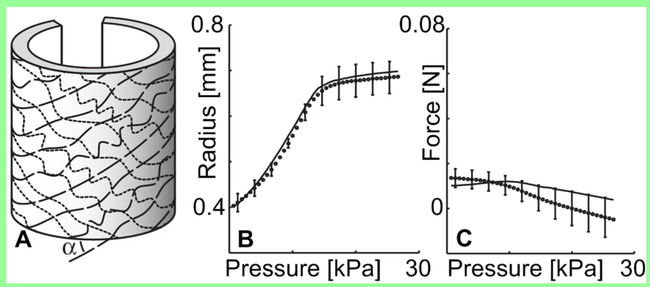



Fig. 3. Comparison of outer radius and axial force (dotted lines )with SEF developed by Zulliger and Fridez (solid line). Zulliger M.A., Journal of Biomechanics 2004 (37) pp. 993 & 997

*Microstructural Hyperelastic Model*

- Zhang developed a microstructural finite-element model to describe the arterial mechanics of the medial layer.
- Elastin crosslinking and structure may be a key method by which the pulmonary artery stiffens with pulmonary hypertension

## Problem Statement:

- To more accurately describe the structure of arterial elastin in terms that are amenable to the formulation of a structural model.

## Methods:

1. Spinning Disk Confocal (SDCM) and 2-Photon Fluorescent Microscopy
   - Will be used to determine the 3-D structure of elastic lamellae through serial images of the x-y plane.
2. Scanning Electron Microscopy (SEM)
   - Used in conjunction with SDCM to describe sub-micron elastic structures
3. Light Microscopy
   - For verification of data generated by SEM and SDCM
4. X-Ray Diffraction
   - To determine the sub-micron molecular orientation of elastic fibers in elastic lamellae.

## Results:

*Typical structure of Elastic Arteries*

- Tunica Intima: Innermost layer consisting of a single layer of endothelial cells and a thin basement membrane.
- Tunica Media: Elastic layer consisting of smooth muscle cells, elastic lamellae and collagen fibrils. The media is also the most important layer for defining arterial mechanics, since it is in this layer that SMCs, collagen and elastin are engaged at physiological stress levers.
- Tunica Adventitia: Helically oriented collagen bundles provide strength and rigidity at high strain levels.

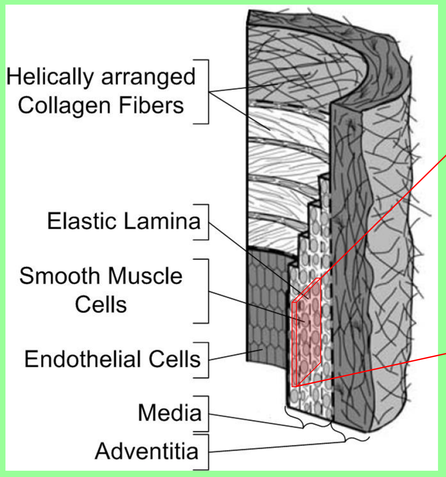
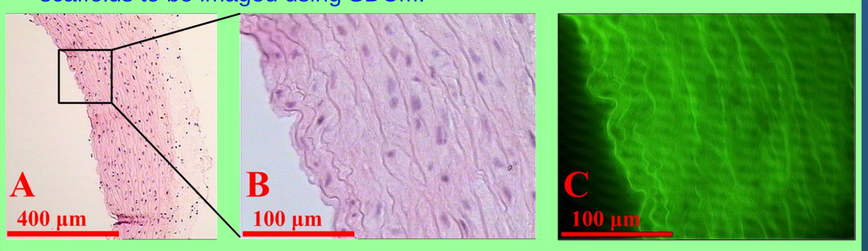



Fig. 4. Typical structure of an elastic artery Holzapfel G.A., Journal of Elasticity 2000 61(1-3) pp. 5

*Brightfield and Epi-Fluorescent Microscopy of H&E stained samples*

- Fig. 5-a & b. Hematoxylin / Eosin stained pulmonary arteries. Cell nuclei stain dark purple, elastin and ECM stain pink. Elastic lamellae appear as dense pink striations between muscle cells.
- Fig. 5-C. Epi-Fluorescent image of the tissue sample in fig 5-b. Eosin Y emission is 520nm, thus allowing for Eosin stained, CNBr –reduced, elastin scaffolds to be imaged using SDCM.




*VVG-stained tissue and SEM*

- Fig. 6-a. Verhoff Van Giesen stained artery. Cell nuclei and elastin stain black, collagen fibers stain red and muscle stains greenish yellow
- Fig. 6-b. Scanning electron micrograph of the intimal and medial layers of an elastic scaffold. The image clearly shows the directionality and overall morphology of the elastic lamellae.

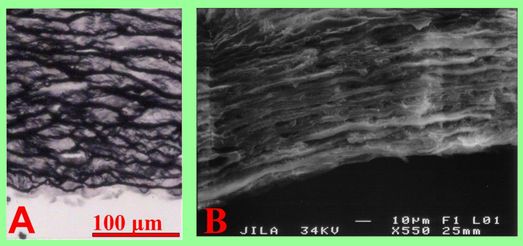



Fig. 6 a) VVG stained artery b) scaffold SEM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*X-Ray Diffraction*

- Used to determine the orientation distribution of elastin molecules in arterial tissues.
- Diffraction pattern varies depending on location within tissue sample
- Analysis is ongoing

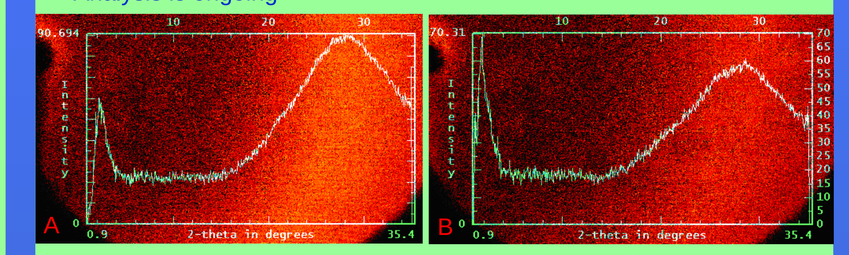


Fig. 7: XRD pattern for A.) Ascending aorta B.) Descending Aorta (Background removed from both images)

*Image Processing*

- An image processing algorithm, written in Matlab, is used to generate a 3-D output from the microscope image stack. Noise is removed with threshold and erosion functions. The resulting binary dataset is then used to generate a 3-D point cloud and rendered image.

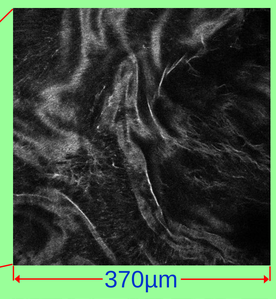


*Fig. 8: Microscope Images*

- 2-photon microscopy is used to image arterial tissues in the radial direction. This allows for sequential imaging through the entire thickness of the sample. The images shown were taken with 40x magnification at 2µm intervals in the z-direction


*Fig. 9: Thresholding*

- A binary image is generated using a threshold algorithm. This allows for the identification of elastic tissue within the sample image. Here, elastin is white and the black regions are those with an image intensity less then the threshold value.


*Fig. 10: Erosion*

- Erosion uses nearest-neighbor properties to identify and eliminate single pixels and small islands from the threshold images, thus reducing signal noise and producing an image more suitable for 3-D rendering and point cloud generation.

*Rendering*

- Eroded images are used to generate a point cloud text file for export to solid-modeling programs. Matlab is also used to produce a rendered image of the processed data.

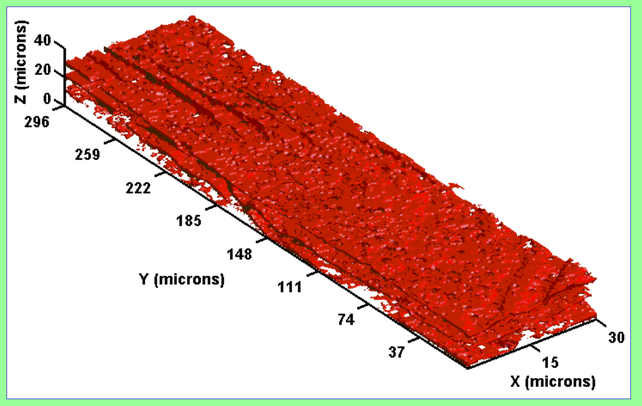


Fig. 11. Rendered Image (31 slices, 2µm separation, Matlab)

## Future Work

- Continue gathering and processing of tissue data for more accurate models of elastic tissues
- Uni-axial stress / strain analysis of elastic tissue scaffolds
- NURB modeling of image stack
- FEA analysis of 3-D models


**1.)** Chuong, C. J. and Y. C. Fung (1983). Journal of Biomechanical Engineering-Transactions of the Asme 105(3): 268-274. **2.)** Holzapfel, G. A., T. C. Gasser, et al. (2000). Journal of Elasticity 61(1-3): 1-48. **3.)** Zulliger, M. A., P. Fridez, et al. (2004). J Biomech 37(7): 989-1000. **4.)** Zhang, Y. H., M. L. Dunn, et al. (2005). "A microstructural hyperelastic model of pulmonary arteries under normo- and hypertensive conditions." Annals of Biomedical Engineering 33(8): 1042-1052.