Quantitative Measures of Collagen Microstructure in Health and Pulmonary Hypertension

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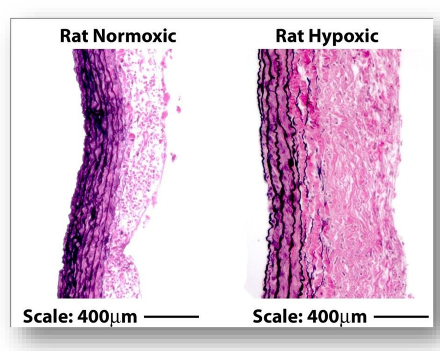
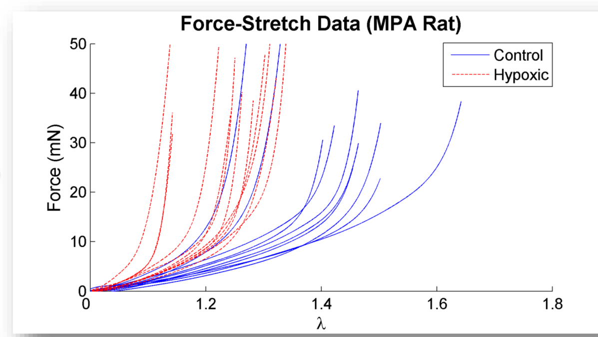
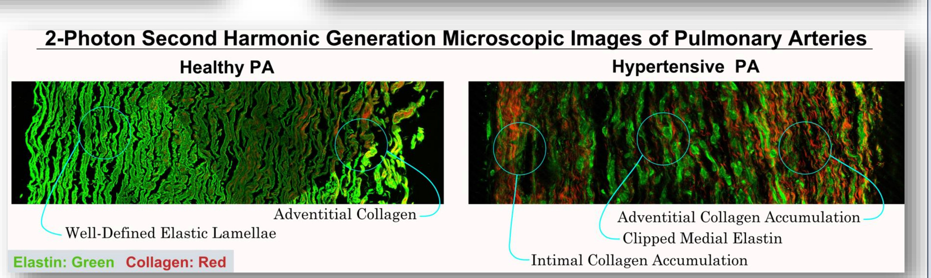
ABSTRACT

- Arterial tissue mechanical non-linearity is largely the result of collagen mechanics.
- Collagen is deposited within arterial tissues as wavy fibrils with significant tortuosity and a bulk fiber orientation of roughly helical configuration. When arterial tissues are deformed, these collagen fibers become straightened in the direction of applied load until at the transition stretch (λ_{Trans}) they are able to carry load, thus significantly altering material stiffness
- We have recently found that λ_{Lock} is significantly reduced in the hypoxia-induced pulmonary hypertensive rat model.





- Significant structural changes occur within collagen and elastin of human extra-pulmonary arteries during pulmonary hypertension



We therefore propose that this rat model constitutes an ideal system to study the effect of collagen microstructure on the mechanics of arterial tissues in response to PH vascular remodeling.

METHODS

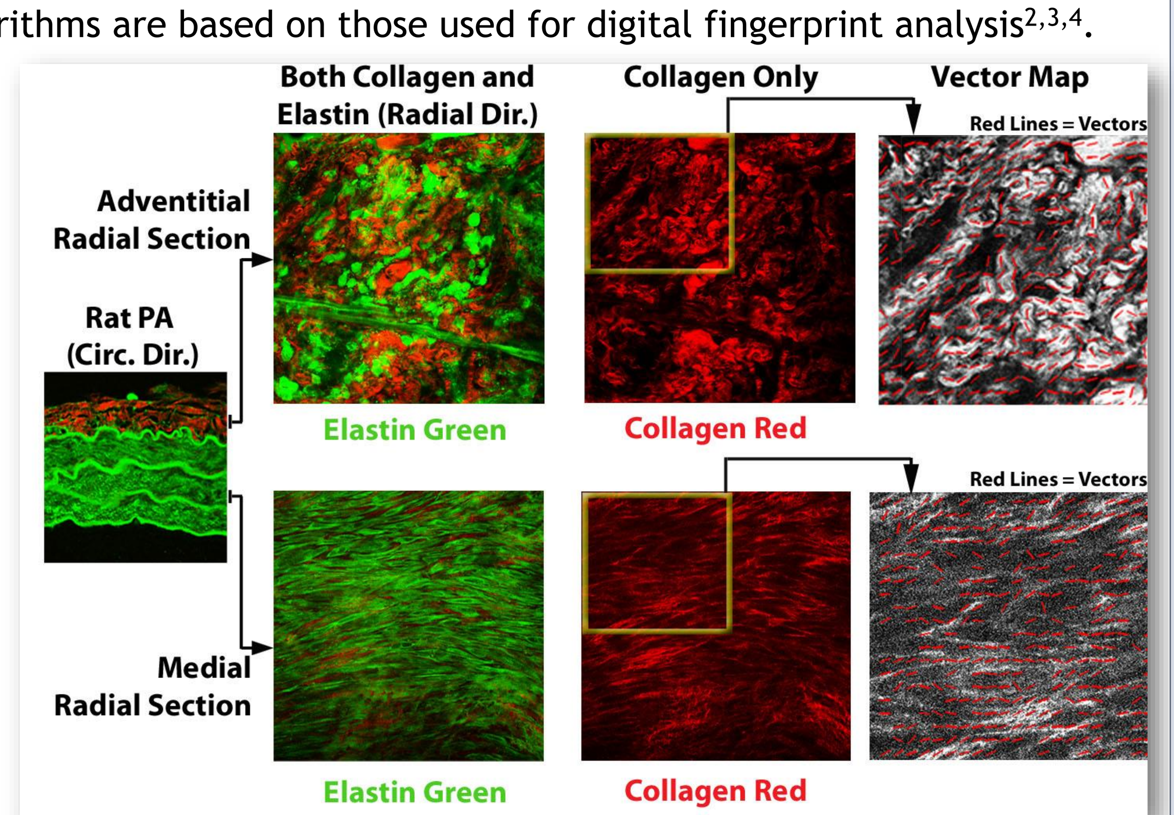
Mechanical Testing: Main pulmonary arteries (MPA) were dissected into circumferential tissue segments and were uniaxially mechanically tested as previously described*,[1].

- Force references the loading region (hysteresis was not considered in this study)
- Collagen λ_{Lock} for mechanical tests was determined based on the change in curvature of the F-λ curve as previously described[1]

Image Processing: Quantitative measurement of global collagen orientation.

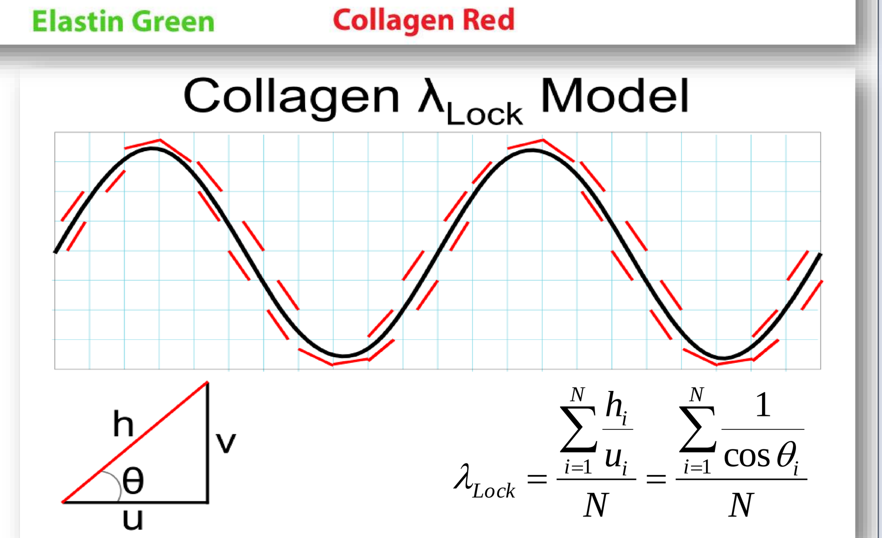
- Images were acquired using 2-Photon Second Harmonic Generation Microscopy.**
  - *Visualize collagen/elastin, label-free, to depths up to 30μm.*
- Custom-written digital image processing functions were implemented in MatLab.
- Image processing algorithms are based on those used for digital fingerprint analysis[2,3,4].

- Sprague-Dawley Rats (12wk old)
- Extra-pulmonary arteries (MPA)
- Images taken at various depths in radial plane (0-20μm)
- Images taken from both the intimal and adventitial sides.
- Images composed of two false-colored intensity images
  - *Collagen red*
  - *Elastin green*



- Collagen channel was isolated for digital image processing resulting in:
  - *Vector map of collagen orientation*
  - *Orientation distribution of collagen was quantified and plotted as histogram.*

Collagen λ_{Lock} Model: Orientation distribution applied to simple model to estimate collagen λ_{Lock}



$$\lambda_{Lock} = \frac{\sum_{i=1}^{N} \frac{h_i}{u_i}}{N} = \frac{\sum_{i=1}^{N} \frac{1}{\cos\theta_i}}{N}$$

HYPOTHESIS

By utilizing digital image processing we will quantify collagen orientation distribution in the radial plane throughout the thickness of extra-pulmonary arterial tissues and use this data to predict the locking stretch at which collagen engages and is able to carry mechanical load within the tissue.

RESULTS

Collagen Orientation Distribution

- Image processing method was validated to be >94% accurate using standardized images of known groups of sinusoidal curves (data not shown).
- Collagen orientation is highly organized throughout the MPA and is highly dependent upon location within the vessel wall.

λ_{Lock} Measured Using Image Data

- Measured from mechanical data: λ_{Lock} = 1.74-1.80 depending on maximum applied load.
- Image-based λ_{Lock} is lower than that measured from mechanical data.
  - Near-intima regions showed significant reduction in λ_{Lock}.
    - Low collagen-density within this region likely mitigates the impact of near-intimal *collagen-mediated* mechanics on the bulk material properties of healthy arterial tissues.
    - The current image processing technique does not fully resolve the high-frequency, low-amplitude, tortuosity of some collagen fibers within this region.

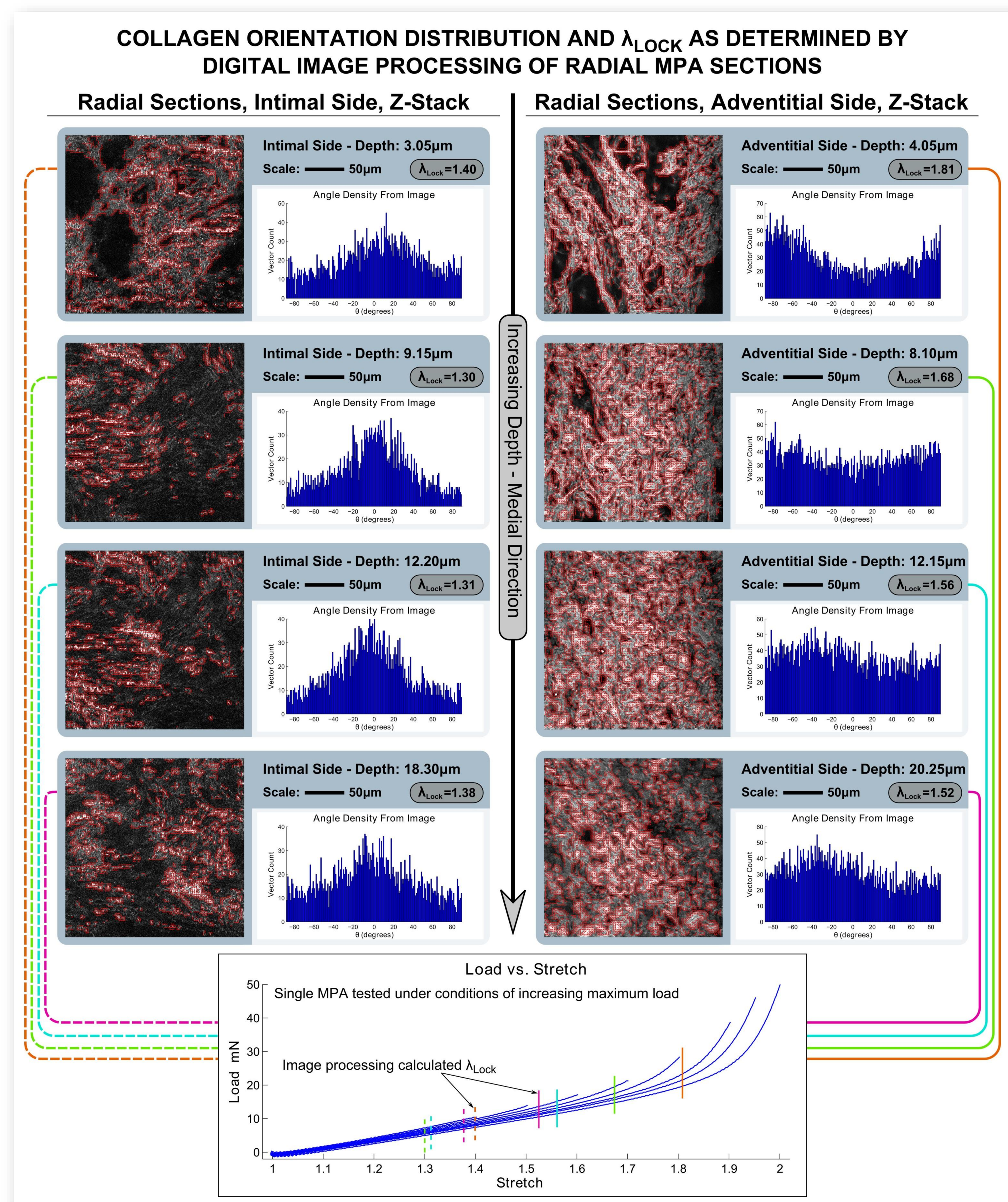


Figure detailing collagen vector maps, orientation distribution plots and locking stretches generated using data obtained from digital image processing. Correlation of image-derived and mechanical results detailed in lower panel. Data generated for single MPA tissue obtained from a healthy adult Sprague-Dawley rat (12wks old).

RESULTS CONTD…

- Near-adventitia λ_{Lock} closely approximates that of mechanical data
  - High collagen-density of this region likely results in significant regulation of collagen-mediated mechanical properties.
  - Collagen λ_{Lock} model will need to be expanded into the third dimension to account for non-radial collagen tortuosity.

CONCLUSIONS

Conclusions

- Using advanced image processing techniques we have shown that the global orientation and tortuosity of collagen can be quantitatively measured throughout the thickness of extra-pulmonary arterial tissues.
- We have further shown that the measured collagen orientation distribution can be used to predict λ_{Lock} from the image data and a simple model of collagen structure.
- These results provide insight into the collagen-mediated artery mechanics directly.
- This experimentally-measured collagen orientation data can be used to eliminate several fitted parameters within existing models of artery mechanics[5,6].
- By more fully understanding the mechanical consequences of arterial collagen deposition we can better elucidate the mechanisms by which the vessels stiffen due to extracellular matrix vascular remodeling resulting from pulmonary hypertension

Limitations: Several limitations exist regarding the implementation of the digital image processing technique used to determine collagen λ_{Lock}.

- Collagen structure is tortuous in both the redial plane (measured here) and the circumferential/longitudinal planes. Incorporation of this additional tortuosity will necessarily lead to larger values of the calculated collagen λ_{Lock}.
- Orientation distribution of collagen is well quantified using the given image processing functions. However, some of the high-frequency small-amplitude structures are not well represented. This will need to be addressed by either further refining the technique or by using images of higher magnification.

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ACKNOWLEDGEMENTS AND FOOTNOTES

Thanks to: Tim Lei PhD.[1], Emily Gibson PhD.[1], Radu Moldovan PhD.[2], Tosin Feyintola[1], Charles Procknow[3]

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Funding Sources: NIH P50HL84923, T32HL072738, SCCOR K24HL081506, PPG HL014985, AHA-09SDG2260194, K25-HL094749, T32-HL007171

Footnotes

* Uniaxial materials testing: MTS Insight 2, 2(N) load cell, isotonic PBS buffer @ 36°C

** Two Photon Second Harmonic Generation Microscope: Zeiss LSM 510 META on Axiovert 200M | Laser: 800 nm | Filters: HQ575/250m-2p, HQ400/20m-2p | Objective: C-Apochromat 40x/1.2

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