CORRELATION OF MECHANICAL PROPERTIES AND MICROSTRUCTURE OF RAT ELASTASE-INFUSION ABDOMINAL AORTIC ANEURYSMS

Mary K. O'Connell[1], Hideo Kimura[2], Eiketsu Sho[2,3], Mien Sho[2,3], Ronald L. Dalman[2,3], Charles A. Taylor[1,3]

(1) Department of Mechanical Engineering
Stanford University
Stanford, CA

(2) Veterans Affairs Palo Alto Health Care System
Palo Alto, CA

(3) Division of Vascular Surgery
Stanford University
Stanford, CA

BACKGROUND

The aortic wall is a highly organized structure composed of a thin intimal layer with endothelial cells, a media of concentric lamellar layers of elastin, collagen, and smooth muscle cells (SMCs), and an outer adventitial layer composed primarily of elastin and collagen. In normal elastic arteries, medial lamellar units are arranged with elastin in thin, concentric, fenestrated sheets. SMCs are circumferentially oriented in concentric layers on both sides of the elastin sheets, attaching via thin elastic fibrils. Concentric sheets of collagen fibers surround each SMC-elastin-SMC lamina, and these sheets of unidirectional fibers are oriented perpendicular to the direction of blood flow within ±45°. In the adventitia, collagen fibers are more clustered, have greater variability in orientation, and attach directly to elastin. These interrelated layers result in nonlinear, anisotropic mechanical properties that enable the vessel to withstand physiological loading conditions.

However, in disease, both mural arterial microstructure and biology change, thereby altering macrostructure and mechanical properties. The etiology of this maladaptive response has not been elucidated. It is known that the microstructure of the abdominal aortic aneurysm (AAA) wall differs significantly from normal aorta with (1) degraded elastic lamellae, (2) reduced quantity and organization of SMCs, (3) increased collagen to elastin ratio, and (4) disorganized collagen remodeling. The identifiable macroscopic effects include mural thinning, dilation, and elongation of the vessel wall, thereby reducing arterial compliance and increasing wall stress.

We have employed a rat elastase AAA model to investigate the correlation between artery mechanical properties and AAA progression. Using a custom mechanical testing apparatus and confocal laser scanning microscopy (CLSM), we have found that the mechanical properties of rat AAA tissue are less compliant and more isotropic than normal tissue. Furthermore, the elastin microstructure changes from fenestrated sheets to discontinuous patches and type I collagen fibers become highly disorganized and non-uniformly separated. We hypothesize that as the aneurysm microstructure changes, the shape evolves from cylindrical to spherical geometry, and due to this increasingly biaxial stress state, its mechanical properties become more isotropic.

METHODS

Abdominal aortic specimens were taken from male Sprague Dawley rats (350-450g). Normal vessels, obtained from Pel-Freez Biologicals (Rogers, AR 72757), were stored at 4°C and thawed within one hour of testing. Aneurysmal vessels were created as in the Anidjar-Dobrin elastase AAA model, resulting in aneurysms of 2-4 times original vessel diameter. Specimens were retrieved two weeks after elastase perfusion, and tested within 2 hours of sacrifice.

Mechanical testing was performed on cylindrical specimens of 1.5-2.5cm in length on a tissue testing device developed in-house. Mechanical testing involved subjecting specimens to two test configurations, with 10 preconditioning cycles prior to each test. The length test involved holding pressure constant at 100 mmHg while varying length from a relaxed state to twice *in vivo* length at a speed of 10.4 cm/min. The pressure test was performed at *in vivo* length as pressure was varied from 0-200mmHg at 5 mL/min infusion rate. Stress was computed from measurements of force obtained using a ten pound load cell (Entran Devices, Inc., Fairfield, NJ 07004) and pressure obtained using a catheter (Millar Instruments, Inc., Houston, TX 77223). Strain measurements were obtained via video images correlated to a standard with circumferential strain obtained through direct diameter measurements and longitudinal strain through UV-illuminated dots applied along the top exterior surface of the tissue.

Degree of isotropy was determined using a ratio of constitutive constants. Fung's exponential strain energy relation [Fung, 1979] was used to correlate tissue stress and strain in both circumferential and longitudinal directions:

$$\psi_o = \frac{C}{2} e^{\left[a_1\left(E_{\theta\theta}^2\right)+a_2\left(E_{zz}^2\right)+2a_4\left(E_{\theta\theta}E_{zz}\right)\right]}$$

A Levenberg Marquardt least squares algorithm was used to compute constitutive constants, and the ratio of a_1/a_2 (termed the isotropy index)

was determined. For isotropic materials, the ratio of a_1/a_2 equals unity, and therefore deviations from unity indicate anisotropy.

Following mechanical testing, tissue specimens were fixed in 4% paraformalin at 100mmHg for 1 hour, and stored in 4% paraformalin until studied. CLSM examination was performed on 30-60 mm thick specimens viewed *en face* from intima to adventitia. The through thickness viewing capability of CLSM was combined with the natural autofluorescent properties of both elastin and collagen (elastin excited at 488nm and emitting at 425nm, collagen and elastin both excited at 805nm and emitting at 515nm) to obtain a 3-dimensional view of collagen and elastin architecture. Collagen structure was separated from that of elastin by subtracting the collagen-elastin combined image from the elastin-alone image.

RESULTS

Normal specimens were found to have typical non-linear, mechanical behavior, and the isotropy index of 0.25 was found. AAA specimens showed non-linear mechanical behavior with increased stiffness, corresponding to higher stress at equivalent strains [Figure 1]. The isotropy index of AAA specimens was closer to unity (1.15), indicating increased isotropy. However, the large variability of these constants masks these changes from significance (±0.83).

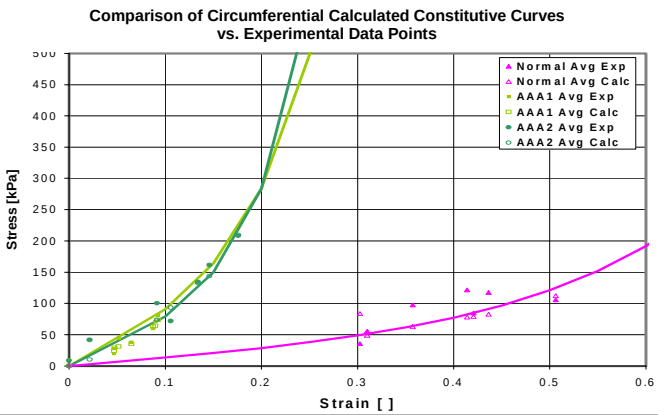

Figure 1 Plot of Stress vs Strain for both normal & aneurysmal rat abdominal aorta specimens. AAA specimens show increased stiffness**.**

Microstructural findings correlate with mechanical findings for both specimens. Normal specimens [Figure 2a] showed definite fibrous sheets of broad collagen type I fibers aligned in a common direction. Fiber direction was dominant in the circumferential direction, but varied from layer to layer, deviating up to 55º from circumferential. Changes in fiber direction was gradual, increasing 5-15º in each successive layer. Fiber orientation is extremely organized in the medial layers and becomes more disorganized, clustered, and branching in the adventitia. Organized, unidirectional sheets of collagen type I fibers in AAA specimens [Figure 2b] were absent, although remnant patches of small fibrous groups were evident. Collagen in AAA specimens appeared to be organized into dense bunches with fiber direction varying within each layer and increasing variability in orientation (0-90º).

DISCUSSION

The passive properties of the artery wall arise in large measure due to the composite structure of elastin and collagen. Elastin enables expansion and retraction under physiological conditions, while collagen provides stiffness to limit dilatation and prevent rupture. The direction of collagen fibers correlates with stress loading under

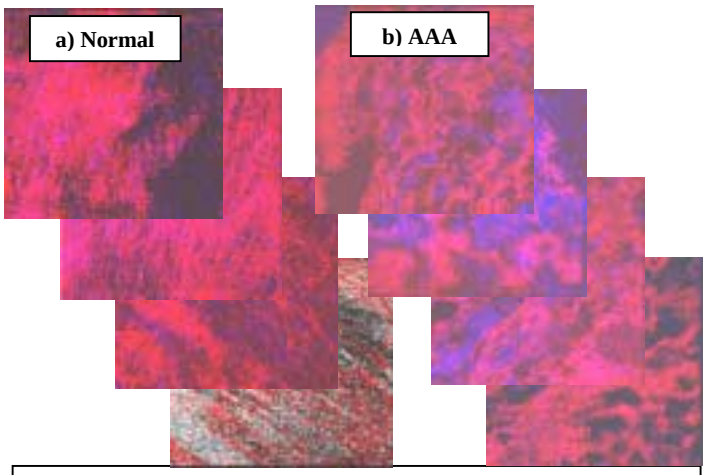

Figure 2 Typical CLSM images of (a) normal and (b) AAA rat aorta showing variations in elastin (blue) and collagen (red) architecture within media (top) and adventitia (bottom). Normal specimen shows organized, unidirectional fiber structure varying in orientation 0-55º). The AAA specimen has no continuous sheets, but rather dense bunches of collagen fibers oriented randomly.

physiological conditions – medial collagen fibers, responsible for supporting circumferential stress, have been recorded as having an average helical collagen fiber orientation of 29º (from circumferential), while adventitial fibers that anchor the vessel have an average orientation of 62º [Holzapfel, 2000].

In this study, mechanical properties were correlated to tissue microstructure for a rat AAA model. Mechanical properties showed that aneurysmal tissue was stiffer and tended toward increased isotropy as compared to normal rat abdominal aorta tissue. The predominantly circumferentially organized collagen fiber sheets observed in normal tissue provide an appropriate composite mechanical structure designed to withstand the dominant pressure loading and minimal longitudinal tension experienced physiologically. The more spherical aneurysmal vessel lacks continuous collagen sheets and organization. Rather, fibers are arranged in dense patches with seemingly random fiber direction. We hypothesize that collagen remodeling during AAA progression lacks circumferential fiber organization due to an increasing biaxial stress state corresponding to a more spherical geometry. Further investigation of the relationship between tissue mechanical properties and microstructure could improve understanding of disease etiology and repair.

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REFERENCES

Anidjar S, Salzmann JL, Gentric D, Lagneau P, Camilleri P, Michel JB. Elastase-induced experimental aneurysms in rats. Circulation 1990;82:973–81.
Fung YC, Fronek K, Patitucci P, *Pseudoelasticity of arteries and the choice of its mathematical expression*, Am. J. Physiol., 237 (5): H620-H631, 1979.
HolzapfelGA, *Biomechanics of Soft Tissue*, Biomech Reprint Series, Paper #7, 2000.