Published in final edited form as:

J Mech Behav Biomed Mater. 2018 January ; 77: 745–753. doi:10.1016/j.jmbbm.2017.08.002.

Transmural Variation in Elastin Fiber Orientation Distribution in the Arterial Wall

Xunjie Yu¹, Yunjie Wang¹, and Yanhang Zhang^{1,2,*}

¹Department of Mechanical Engineering, Boston University, Boston, MA

²Department of Biomedical Engineering, Boston University, Boston, MA

Abstract

The complex three-dimensional elastin network is a major load-bearing extracellular matrix (ECM) component of an artery. Despite the reported anisotropic behavior of arterial elastin network, it is usually treated as an isotropic material in constitutive models. Our recent multiphoton microscopy study reported a relatively uniform elastin fiber orientation distribution in porcine thoracic aorta when imaging from the intima side (Chow et al., 2014). However it is questionable whether the fiber orientation distribution obtained from a small depth is representative of the elastin network structure in the arterial wall, especially when developing structure-based constitutive models. To date, the structural basis for the anisotropic mechanical behavior of elastin is still not fully understood. In this study, we examined the transmural variation in elastin fiber orientation distribution in porcine thoracic aorta and its association with elastin anisotropy. Using multi-photon microscopy, we observed that the elastin fibers orientation changes from a relatively uniform distribution in regions close to the luminal surface to a more circumferential distribution in regions that dominates the media, then to a longitudinal distribution in regions close to the outer media. Planar biaxial tensile test was performed to characterize the anisotropic behavior of elastin network. A new structure-based constitutive model of elastin network was developed to incorporate the transmural variation in fiber orientation distribution. The new model well captures the anisotropic mechanical behavior of elastin network under both equi- and nonequi-biaxial loading and showed improvements in both fitting and predicting capabilities when compared to a model that only considers the fiber orientation distribution from the intima side. We submit that the transmural variation in fiber orientation distribution is important in characterizing the anisotropic mechanical behavior of elastin network and should be considered in constitutive modeling of an artery.

Keywords

Elastin; anisotropy; constitutive model; transmural variation; biaxial tensile testing; multiphoton microscopy; fiber orientation distribution

*Corresponding author: Yanhang (Katherine) Zhang, Department of Mechanical Engineering, Department of Biomedical Engineering, Boston University, 110 Cummington Mall, Boston, MA 02215, yanhang@bu.edu, Phone: (617)358-4406, Fax: (617)353-5866.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Elastin is one of the major extracellular matrix (ECM) components that imparts elastic property to an artery in order to accommodate cyclic physiological deformation. In elastic arteries such as aorta, elastin fibers in the medial layer form concentric layers of elastic lamellae. Each elastic lamella alternates with a layer of smooth muscle cells and collagen fibers forming a lamellar unit, which is considered as a functional unit of the arterial wall (Wolinsky & Glagov, 1967). It is believed that elastin dominates the passive behavior of arteries at low strains whereas collagen are progressively recruited at higher strains (Roach & Burton, 1957). Using multi-photon microscopy, Chow et al. (Chow et al., 2014) quantified the sequential engagement of elastin and collagen fibers in response to arterial deformation.

Blood vessels are generally considered to be anisotropic (Zhou & Fung, 1997). Various forms of strain energy function have been developed, however the contribution of elastin to arterial wall mechanics is usually assumed to take isotropic forms, and the anisotropic response of arterial tissue usually comes from preferred collagen fiber distribution. (Wuyts et al., 1995) (Holzapfel et al., 2000) (Fung & Liu, 1989) (Zulliger et al., 2004) (Zeinali-Davarani et al., 2013). Several recent studies attempt to account for the anisotropic mechanical properties of elastin. Rezakhaniha and Stergiopulos (Rezakhaniha & Stergiopulos, 2008) considered a model with one family of axially oriented fibers embedded in an isotropic matrix to model elastin as a transversely isotropic material. Zou and Zhang (Zou & Zhang, 2009) developed a statistical mechanics-based hyperelastic anisotropic constitutive model to study purified elastin network. Kao et al. (Kao et al., 2011) used an orthotropic representation that consists of two orthogonal families oriented in the axial and circumferential directions distributed in a matrix. Rezakhaniha et al. (Rezakhaniha et al., 2011) assumed a model with elastin fibers oriented in the circumferential direction and a better model predictability was reported. Wang et al. (Wang et al., 2016) considered the unique contribution of elastin, medial collagen, and adventitial collagen fibers in a structure-based constitutive model of the arterial wall that incorporates fiber orientation and engagement of each ECM components (Wang et al., 2016).

Natural biological tissues and tissue-derived soft biomaterial often exhibit pronounced mechanical anisotropy due to preferred fibrous orientation (Sacks, 2000). Ligaments and tendon have anisotropic behavior with a preferred direction that can be represented by transverse isotropy (Hoffmeister et al., 1996) (Ono et al., 1990) (Weiss et al., 1996). Fiber structure revealed from small angle light scattering technique was found to be consistent with the direction and the degree of mechanical anisotropy of canine diaphragmatic central tendon (Chuong et al., 1991). Canine myocardium exhibits anisotropic behavior in the along-fiber and cross-fiber directions (Demer & Yin, 1983). Elastin network has been observed to have anisotropic mechanical behavior (Sherebrin et al., 1983) (Zou & Zhang, 2009) (Lillie et al., 2010). The structural basis for the anisotropic mechanical behavior of elastin, however, is still not clear. In this study, we focus on the transmural variation in elastin fiber orientation distributions and the association with the anisotropic behavior of elastin network. Multiphoton microscopy was used to image purified medial elastin network from both sides as well as sections from various depths to capture the transmural variation in

elastin fiber distribution. Equi- and nonequi-biaxial tensile tests were performed on purified elastin network to characterize its mechanical behavior. Finally a structure-based constitutive model was developed to incorporate the measured elastin fiber distributions from the inner, middle, and outer media for the mechanical behavior of elastin network.

Material and Methods

Sample preparation

Descending thoracic aortas were harvested from pigs of 12–24 month of age at a local abattoir and transported to laboratory on ice. After removing of adhesive tissue and fat, samples of approximately 20 × 20mm square were cut with one edge parallel to the longitudinal direction and the other edge parallel to the circumferential direction of the artery. Purified elastin was obtained using a cyanogen bromide (CNBr) treatment to remove cells, collagen and other ECM components (Zou & Zhang, 2009). Aorta samples were kept in 50 mg/ml CNBr in 70% formic acid at room temperature for 19 h, then at 60 °C for 1 h with gentle stirring, followed by boiling for 5 min to inactivate CNBr. Samples were rinsed in DI water and 1 × phosphate buffered saline (PBS) several times and placed in PBS for mechanical testing and imaging.

Mechanical testing

Equi- and nonequi-biaxial tensile tests were performed using a biaxial tensile testing device to characterize the anisotropic mechanical properties of elastin network following protocols described previously (Zou & Zhang, 2009). A total of six samples were tested. Briefly, sandpaper tabs were glued to the edge of the samples, and nylon sutures were looped through the sandpaper tabs and then connected to linear carriages. Tension control experiments were performed through a custom LABVIEW program. There were two load cells, one in each axis, to monitor the load applied to the tissue sample in both loading directions. The position of the four carbon marker dots glued to samples was monitored using a CCD camera to measure tissue deformation. A preload of 5 N/m was applied to the samples in order to straighten the sutures. Samples were subjected to eight cycles of equi-biaxial tension of 40 N/m for preconditioning. Following preconditioning, eight cycles of biaxial tension with $f_l:f_c = 100:100$, $100:75$, and $75:100$ N/m were applied to achieve repeatable mechanical response, where f_l and f_c refers to tension in the longitudinal and circumferential directions, respectively. Here tension is calculated as force divided by the side length of the sample where the force is applied. Measurements from the last cycle were used for data analysis. Cauchy stresses were calculated by assuming plane stress and incompressibility as:

$$\sigma_1 = \frac{F_1 \lambda_1}{t L_{20}}, \quad \sigma_2 = \frac{F_2 \lambda_2}{t L_{10}} \quad (1)$$

where σ_i is Cauchy stress, F_i is the applied load, L_{i0} is the initial side length, and t is the initial thickness of the tissue. λ_i is stretch, which was calculated as $\frac{L_i}{L_{i0}}$, where L_i is the

deformed side length. Subscripts $i = 1, 2$ correspond to the longitudinal and circumferential directions, respectively.

Multiphoton microscopy

A multiphoton microscopy system (Carl Zeiss LSM 710 NLO) with a 810 nm femtosecond IR pulse laser excitation was used to generate two-photon excited fluorescence (2PEF) from elastin (525/45 nm). Laser power at the sample was set to 25 mW to minimize thermal effects. The laser scanning system is coupled to an upright microscope with a $20\times$ water immersion objective lens. Each sample was imaged with a field view of $425 \times 425 \mu\text{m}$ at five locations to obtain average structural properties of the samples. A total of six samples were imaged. Samples were placed with longitudinal direction of the tissue aligned vertically. Thus, fibers oriented at 0° and $\pm 90^\circ$ are in the circumferential (C) and longitudinal (L) directions, respectively. Samples were imaged from both sides to assess the inner and outer medial elastin, which was referred to as inner media and outer media throughout the study. Regions between the inner and outer media were referred to as middle media. A custom-built device was used that allows the elastin samples to be imaged while under biaxial strain (Chow et al., 2014). The samples were imaged from the intimal side when subjected to up to 30% equi-biaxial strain at 10% increments. To examine elastin fiber reorientation under nonequi-biaxial deformation, samples were imaged at strains of 0%C-0%L, 30%C-30%L, 15%C-30%L, 30%C-15%L, where the strains represent grip-to-grip engineering strain. The elastin samples were then frozen, and from $400 \mu\text{m}$ beneath intima surface, three $\sim 100 \mu\text{m}$ thick sections were cut in parallel to the intima surface using a microtome (MICROM cryostat HM 525). The sections were collected on slides for imaging. Samples were imaged to a depth of about $40 \mu\text{m}$ with scans every $2 \mu\text{m}$. Maximum intensity projections of the Z-stacks were produced for further analysis. Sections of about $100 \mu\text{m}$ in thickness were also prepared to image the circumferential cross-sectional structure of the elastin network.

Imaging analysis

Two dimensional fast Fourier transform (2D-FFT) analysis using the Directionality plug-in (developed by Jean-Yves Tinevez) in FIJI (<http://Fiji.sc/Fiji>, Ashburn, VA) was performed on 2PEF images to determine fiber orientation distribution, following developer's instructions. The fiber orientation in the spatial frequency space was determined and a normalized histogram was generated to represent the amount of fibers at angles from -90° to 90° at 2° increment (Chow et al., 2014). Each sample was imaged at five locations that cover about 1cm^2 area. Average fiber orientation distributions from six samples, or 30 locations, were obtained and used for constitutive modeling.

The fiber orientation ratio of circumferentially to longitudinally oriented fibers, defined as the number of circumferential fibers (oriented between $0^\circ \pm 20^\circ$) divided by the number of longitudinal fibers (oriented between $90^\circ \pm 20^\circ$), was calculated to compare the degree of fiber alignment.

Constitutive modeling

To account for transmural variation in elastin fiber orientation distribution, the elastin network was modeled using a constitutive model that incorporates the orientation

distributions of elastin fibers $R_i(\theta)$ ($i = im, mm, om$ correspond to the inner, middle, and outer medial elastin, respectively) at different transmural depths. The total strain energy function is the sum of strain energy function at the inner, middle, and outer medial elastin and can be written as:

$$W = \sum_{i=1}^3 n_i \int_{-\pi/2}^{\pi/2} w(\rho) R_i(\theta) d\theta \quad (2)$$

where $w(\rho)$ is the strain energy function at the fiber level, and n_i is the elastin content of each elastin region (Wang et al., 2016). The elastin fiber is model as an entropy-based freely-jointed chain, based on non-Gaussian statistics for large deformation, the strain energy function can be written as (Kuhn & Grn, 1942):

$$w(\rho) = k\Theta N \left(\frac{\rho}{N} \beta_\rho + \ln \frac{\beta_\rho}{\sinh \beta_\rho} \right) \quad (3)$$

where N is the number of rigid links within each chain, ρ is the normalized deformed chain length and is related to fiber-level Green–Lagrange strain ε by $\rho = P \sqrt{2\varepsilon + 1}$, and $P = \sqrt{N}$ is the normalized undeformed chain length. $\beta_\rho = \mathcal{L}^{-1}(\rho/N)$, where $\mathcal{L}(x) = \coth x - 1/x$ is the Langevin function. $k = 1.38 \times 10^{-23} \text{ J/K}$ is Boltzmann’s constant, and $\Theta = 298 \text{ K}$ is the absolute temperature.

For comparison, elastin network was also modeled using a constitutive model that only incorporates the fiber orientation distribution from the inner media (Wang et al., 2016), which does not consider the transmural variation in fiber orientation distribution. The strain energy function of the elastin network in this case can be written as:

$$W = n \int_{-\pi/2}^{\pi/2} w(\rho) R_{im}(\theta) d\theta \quad (4)$$

The Cauchy stress can then be obtained from $\boldsymbol{\sigma} = J^{-1} \mathbf{F} \mathbf{S} \mathbf{F}^T$, where $\mathbf{S} = W/\mathbf{E}$ is the second Piola–Kirchhoff stress, $\mathbf{F}$ is the deformation gradient, and $J = \det(\mathbf{F})$. In the freely-jointed chain model (Equation 3), material parameter N is related to the length of molecular chains between cross-links at the micro-level. It dictates the extensibility of the material at the tissue level. A more extensible material is expected to be less cross-linked and thus has a larger N . In Equations 2 and 4, material parameter n is the chain density per unit volume, and is related to the elastin content, and the initial stiffness of the material. More detailed discussions on the physical meanings of the material parameters and their interdependence in determining the tissue mechanical properties can be found from (Zhang et al., 2005) (Zhang et al., 2007).

Parameter estimation

Material parameters were estimated by minimizing the difference between the computed Cauchy stress from constitutive models, σ^c and the stress obtained from the experiment, σ^e , in the form of the following objective function,

$$E = \sum_{i=1}^k [(\sigma_{11}^c - \sigma_{11}^e)^2 + (\sigma_{22}^c - \sigma_{22}^e)^2] \quad (5)$$

where k is the number of data points, and subscripts 1 and 2 correspond to the longitudinal and circumferential directions of the sample, respectively. The objective function is minimized using the Nelder-Mead direct search method implemented in the *fminsearch* function in Matlab (version R2013b, The MathWorks, Inc.) (Zeinali-Davarani et al., 2015).

The proposed model has four material parameters: n_i ($i=im, mm, om$), and N . A penalty approach was employed to reinforce constraints and to ensure the model parameters have physiological meanings. Based on the image of the cross sections of elastin network (Figure 1), the content of elastin that forms the inner and outer media was assumed to be much smaller than the content of elastin from the middle media, therefore, we assumed that

$$0.05n < n_{im}, n_{om} < 0.25n \quad (6)$$

The model that only incorporates the fiber orientation distribution from the intimal side has two material parameters, n and N . In addition, it was also assumed that $N > 1$. To determine the material parameters, the stress-stretch responses from the model were fit to the biaxial-tensile testing data. To assess the fitting and predicting capabilities of the model, we first fit the model to all three sets of experimental data with biaxial tension $f_1:f_c = 100:100, 100:75$, and $75:100$ N/m. We then fit the model to two sets of nonequi-biaxial tension $f_1:f_c = 100:75$ and $75:100$ N/m, and use the obtained model parameters to predict the mechanical behavior of elastin network under equi-biaxial tension $f_1:f_c = 100:100$ N/m. To measure of the goodness of fitting and predication, the root-mean-square (RMS) measure of error is defined as (Holzapfel et al., 2005):

$$e = \frac{\sqrt{\frac{E}{m-q}}}{\sigma_{ref}} \quad (7)$$

where q is the number of parameters in the model, m is the number of data points, E is the objective function defined by Equation (5), and σ_{ref} is the sum of experimental Cauchy stresses for each stress-stretch curve divided by the number of data points m .

Statistical analysis

Experimental data were presented as mean $\pm$ standard error of the mean (SEM). The ratios of circumferential to longitudinal fibers at various depths were compared using one-way analysis of variance. $p < 0.05$ is considered statistically significant with post hoc testing using the Tukey's method to adjust for multiple comparisons (Statistical analysis was performed using the JMP statistical package.)

RESULTS

Elastin fibers form concentric layers of elastic lamellae in the medial layer of the arterial wall, as shown in the circumferential cross-sectional 2PEF images in Figure 1. The images reveal transmural changes in the organization of elastin fibers within the elastic lamella. Elastin fibers close to the inner (inner media) and outer (outer media) surface of the medial elastin appear as discontinuous circular dots, implying the fibers are aligned longitudinally. Within the middle media, i.e., regions between the inner and outer media, elastin fibers appear as continuous lines, implying the fibers are aligned circumferentially. It is also apparent that the inner and outer media is much thinner compared to the middle media.

The transmural variations in the distribution of elastin fibers are further revealed by 2PEF images taken at various depths through the thickness of the sample. As shown in Figure 2, images taken from the inner and outer media, i.e., locations 1 and 5, show obvious differences from images taken from the middle media, i.e., locations 2, 3 and 4. The fiber orientation distributions were quantified with results shown in Figure 3. In inner media, elastin fibers are relatively uniformly distributed with slightly more fibers aligned in longitudinal direction, as shown by the appearance of small peaks toward $\pm 90^\circ$ in Figure 3a. In middle media, elastin fibers are dispersed around the circumferential direction. In outer media, elastin fibers uniformly align in the longitudinal direction. The fiber orientation ratios of circumferentially to longitudinally oriented fibers are 0.6405 ± 0.1109 , 3.1537 ± 0.3926 , and 0.2248 ± 0.0339 for inner, middle, and outer media, respectively. There was a significant transmural variation in fiber orientation distributions between inner and middle media, as well as between middle and outer media ($p < 0.05$).

Representative multiphoton images of elastin network at inner media during equi-biaxial deformation were shown in Figure 4. Elastin fibers appear to be straight even when unloaded, and don't show obvious straightening or reorientation under biaxial strain up to 30%. FFT analyses of elastin fiber orientation distribution under equi- and nonequi- biaxial strain are shown in Figure 5. Elastin at inner media does not appear to have a change in fiber orientation in respond to biaxial deformation. Although not shown here, no significant changes were observed in fiber orientation ratio of circumferential to longitudinal fibers form both equi- and nonequi- biaxial loading.

In order to explore whether the elastin purification process causes any changes in the structure of elastin network, in Figure 6 we compared the fiber orientation distribution of elastin fibers in the purified elastin network with that in the arterial tissue (Chow et al., 2014). There is a slight increase in the longitudinally oriented fibers in the purified elastin network, however overall the fibers remain uniformly distributed. Further quantification of

the ratio of circumferentially to longitudinally distributed fibers shows no significant difference in elastin fiber distribution between purified elastin and artery.

Figures 7 and 8 show the representative stress-stretch responses of elastin under equi- and nonequi-biaxial tension as well as results from constitutive modeling. In Figure 7, three sets of experiment data (one equi-biaxial and two nonequi-biaxial tension, $f_l:f_c = 100:100$, $100:75$, and $75:100$ N/m) were fitted simultaneously. In Figure 8, the model was fit to two sets of nonequi-biaxial tension data and predications of the stress-stretch behavior under equi-biaxial tension were made to assess the model predictability. To quantify elastin anisotropy, the circumferential and longitudinal stretches at 80kPa from equi-biaxial tension were compared for the six samples tested. The circumferential stretch is significantly lower than the longitudinal stretch (1.0747 ± 0.0082 vs. 1.1181 ± 0.0114 , $p < 0.05$), implying that the elastin network is stiffer in the circumferential direction.

The anisotropic mechanical behavior of elastin is well described by the structure-based model that considers the transmural variation in elastin fiber distribution (Figures 7a and 8a). However only accounting for elastin fiber orientation distribution from the inner media results in poor fitting capabilities (Figures 7b and 8b). Tables 1 and 2 summarize the model parameters, and fitting and predicting errors. When fitting three sets of experiment data, the model that only considers the elastin fiber orientation distribution from the inner media shows an average RMS (root mean square) error of 0.3940 ± 0.0550 , which is significantly higher than 0.1103 ± 0.0112 , when the transmural variation in elastin fiber distribution is considered ($p < 0.05$). Similar RMS for each model was obtained when fitting two sets of nonequi-biaxial tension data. The predictive capability is also significantly improved in when considering the transmural variation with RMS being 0.1311 ± 0.0085 compared to 0.4018 ± 0.061 when only the elastin fiber orientation distribution at inner media was considered ($p < 0.05$). The estimated value n_{mm} for middle media elastin content was nearly one order of magnitude higher than n_{im} and n_{om} , indicating that the stress-stretch contribution of middle medial elastin is much higher than inner and outer medial elastin.

DISCUSSION

Altered elastin network is associated with vascular remodeling processes in many pathological conditions (Wagenseil et al., 2009)(Maiellaro-Rafferty et al., 2011). Understanding the organization of elastin fibers in arteries and its connection with the mechanical behavior of an artery is essential for understanding the mechanisms of vascular remodeling. Our study shows there is a unique transmural variation in the orientation distribution of elastin fibers through the medial layer of the arterial wall. A structure-based constitutive model was created to incorporate the transmural variation of elastin fiber distribution. The model showed improved fitting and predicting capabilities when compared to the model that only considers fiber orientation distribution from the inner media. This is the first study that transmural structural inhomogeneity is considered in order to understand the structure and anisotropic mechanical function of elastin network in the arterial wall.

Multiphoton imaging at multiple transmural locations reveals that the orientation of elastin fibers changes from relatively uniformly aligned in the inner media to circumferentially

aligned in the middle media, then to longitudinally aligned in the outer media (Figure 3). The middle media appears to be much thicker than the inner and outer media (Figure 1). Alternations in elastin fiber orientation in the arterial wall was reported earlier from studies using electron microscopy (Clark & Glagov, 1985) (Farand et al., 2007). It was also observed that the smooth muscle cells follow a similar transmural distribution as the elastin fibers (Clark & Glagov, 1985). This distinct transmural variation in fiber orientation helps to accommodate the complex loading in the artery. The axially oriented fibers from the intima side support the shear stress from blood flow in the longitudinal direction, while the thicker middle media elastin oriented in the circumferential direction bears the pulsatile load in the circumferential direction (Clark & Glagov, 1985) (Farand et al., 2007). Axially aligned elastin fibers in the outer media, however, play a role in transmitting axial shear stress from organ movement and tethering by adventitia and branches to media (Clark & Glagov, 1985).

The elastin in this study are purified medial elastin network obtained from CNBr treatment, which involves the removal of collagen fibers, cells, proteoglycans, etc. (Zou & Zhang, 2009). As elastin fibers are dispersed in the adventitia in the form of randomly oriented fiber strands (Fata et al., 2013), it is expected that elastin fibers in the adventitia were removed along with the removal of adventitial collagen. The elastin fibers in the medial layer form dense concentric layers of elastic lamellae. If any adventitial elastin fibers are present, they should be easily distinguished. Figure 7 shows that the elastin fibers in the purified elastin network are slightly more longitudinally orientated than the elastin fibers in the intact artery. This may result from the removal of predominantly circumferentially oriented medial collagen fibers (Chow et al., 2014).

The structural basis of the anisotropic mechanical behavior of elastin is not very well understood. Previous study ascribed the anisotropy of elastin network to fiber reorientation (Ronchetti et al., 1998) (Rezakhaniha & Stergiopulos, 2008) and the intralamellar elastin fibers orientating in the circumferential direction (Rezakhaniha et al., 2011). Our study suggested that the elastin fiber network does not show obvious structural changes under deformation (Figures 4 and 5). Similar behavior were shown by Chow et al. (Chow et al., 2014) and Fata et al. (Fata et al., 2013) of elastin fibers in intact arteries. Timmins et al. (Timmins et al., 2010) suggested that the nonlinear, anisotropic mechanical response for vascular tissue is a direct result of fiber orientation. Using Multiphoton microscopy, elastic fibers in large arteries were reported to have a relative uniform distribution (Chow et al., 2014) (Fata et al., 2013). It is important to note here that due to laser penetration limits, imaging of elastin network was achieved from the intimal side of the arterial wall with a maximum depth of 200 μm (Chow et al., 2014) (Timmins et al., 2010) (Fata et al., 2013), which is very small compared to the arterial wall thickness (~ 1.5 mm). It is thus questionable whether the fiber distribution obtained from such a limited imaging depth from the intimal surface is representative of the tissue structure. This is especially important when studying the alterations in ECM structures, and when developing structure-based constitutive models.

In structure-based models, fiber orientation distribution usually is the only source of tissue anisotropy. This study, however, provides further understandings on the origin of the anisotropic behavior of elastin network. Besides fiber orientation distributions, transmural

variation in fiber orientation distributions, and the proportions of the inner, middle and outer media regions in elastin play an important role in determining tissue anisotropy. The dominated circumferentially oriented elastin fibers (Figure 1) explain well with the generally stiffer behavior in the circumferential direction of the elastin fiber network (Figures 7 and 8). Although not shown, we also found the fiber orientation distributions in the inner, middle, and outer media do not vary much among the samples. Previous studies (Lillie & Gosline, 2007) (Zou & Zhang, 2009) reported that the mechanical behavior of elastin network becomes increasingly anisotropic along the aorta from proximal to distal end. Such observation was speculated to be the result of more circumferentially oriented fibers in the distal tissue. It would be interesting to examine whether this regional variation in the mechanical behavior of elastin is related to the changes in the proportions of inner, middle and outer media along the aorta. Further, alterations in wall stresses have been reported to lead to alterations in wall thickness, composition and structural organization of medial elastin and collagen. (Rodbard, 1970) (Glagov et al., 1988) (Ku, 1997). Low wall shear stress has been reported to cause thickening of the intimal layer (Glagov et al., 1988). The transmural organization of elastin fibers and its association with arterial remodeling is unknown.

Experimentally measured structural parameters from imaging, such as fiber orientation distribution, fiber angles, fiber crimping, and constituent volume fractions, can be incorporated directly into structure-based constitutive models to reduce the number of parameters and improve prediction accuracy (Hill et al., 2012) (Wang et al., 2016). By incorporating experimentally measured collagen fiber orientation distribution into the constitutive models, improvement in the predicting capability of the model was achieved without significant loss in the goodness of fit (Wan et al., 2012). The transmural fiber orientation distribution of collagen, another major ECM constituent, has been found to change from nonuniform distribution in subendothelial layer to circumferentially oriented in the media, and to helically arranged fiber families in the adventitia (Gasser et al., 2006). The small artery size allows measurements of transmural collagen fiber orientation distribution through multiphoton microscopy. The experimentally measured collagen fiber orientations through the thickness of mouse carotid arteries was considered by using a four-fiber family model (Wan et al., 2012).

While there are wide studies on structurally-based models considering collagen fiber microstructural organization (Wan et al., 2012), only a few models attempted to incorporate elastin network anisotropy. By considering an anisotropic strain energy function for elastin in the artery, better fitting of mechanical behavior of artery was achieved (Rezakhaniha & Stergiopulos, 2008) (Rezakhaniha et al., 2011). A model with anisotropic strain energy function for elastin may offer a better description of the biomechanical behavior of arterial wall (Rezakhaniha & Stergiopulos, 2008). Considering the transmural variation in medial elastin, the directly measured fiber orientation distributions from inner, middle and outer media were incorporated into the structure-based constitutive model (Figure 3). Since the change of fiber orientation with deformation was not significant (Figures 4 and 5), fiber orientation distribution at unloaded state (0%C-0%L) was employed, and realignment of elastin fibers under mechanical loading was not considered in our model. By considering the

transmural elastin fiber distribution, the structure-based model showed improved fitting and predicting capability (Tables 1 and 2).

LIMITATIONS

Multiphoton imaging has emerged as a promising approach to study and quantify the structural of ECM fibers with minimal sample preparation. However the imaging depth is limited by laser penetration, and thus interpretation of the results should be conducted carefully. Fiber structure during mechanical loading examined in this study is limited to elastin fibers from the inner media. Changes in fiber orientation distribution in the middle and outer media are unknown. Material parameter N is assumed to be the same for inner, middle, and outer media. It is possible that the molecular arrangement of elastin is different in different locations so that N would be different in each region. Material parameters that produce the best fit to the stress-stretch curve are generally not unique. This is a common limitation of the *fminsearch* function in MATLAB that provides a solution satisfying the local minimum. The imaging results provide guidance on constraints to model parameters (Equation 6), and ensure material parameters to be obtained within a physiologically meaningful range. However even with the defined constraint, the solutions may still not be unique as the initial guess plays an important role as well. While it is obvious that the middle media is much thicker than the inner and outer media; it is difficult to quantify the thickness of each region from the cross-sectional view in Figure 1. Thus the elastin content in each region was kept as a free parameter, but satisfied a constraint defined by Equation 6. However please note that this constraint were not strictly measured and could differ with location, tissue types, and species.

CONCLUSIONS

Our study shows there is a transmural variation in the orientation distribution of elastin fibers through the medial layer of the arterial wall. The orientation of elastin fibers changes from slightly longitudinally aligned in the inner media to circumferentially aligned in the middle media, then to longitudinally aligned in the outer media. Considering the transmural structural inhomogeneity is important when understanding the structure and function of elastin network in the arterial wall. Our study suggests fiber orientation is the main source of elastin anisotropy, which arises from the dominated circumferentially orientated elastin fibers in the middle media. The transmural variation in fiber orientation distributions, and the proportions of the inner, middle and outer media regions in elastin needs to be considered in structure-based constitutive modeling of elastin network for improved fitting and predicting capabilities. The new model well captures the anisotropic behavior of elastin network during equi- and nonequi-biaxial mechanical loading. The current study provides new understandings on the transmural variations in elastin fiber distribution, which is essential in accommodating the complex loading in the artery, and should be included in constitutive modeling to study the mechanical properties of arteries in health and diseases.

Acknowledgments

This work was supported, in part, by a grant (CMMI 1463390) from National Science Foundation, and a grant (R01HL 098028) from National Institutes of Health.

References

- Chow MJ, Turcotte R, Lin CP, Zhang Y. Arterial extracellular matrix: A mechanobiological study of the contributions and interactions of elastin and collagen. *Biophysical Journal*. 2014; 106(12):2684–2692. <https://doi.org/10.1016/j.bpj.2014.05.014>. [PubMed: 24940786]
- Chuong CJ, Sacks MS, Johnson RL, Reynolds R. On the anisotropy of the canine diaphragmatic central tendon. *Journal of Biomechanics*. 1991; 24(7):563–576. [https://doi.org/10.1016/0021-9290\(91\)90289-Y](https://doi.org/10.1016/0021-9290(91)90289-Y). [PubMed: 1880140]
- Clark JM, Glagov S. Transmural organization of the arterial media. The lamellar unit revisited. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 1985; 5(1):19–34. <https://doi.org/10.1161/01.ATV.5.1.19>.
- Demer LL, Yin FC. Passive biaxial mechanical properties of isolated canine myocardium. *The Journal of Physiology*. 1983; 339(1):615–630. [PubMed: 6887039]
- Farand P, Garon A, Plante GE. Structure of large arteries: Orientation of elastin in rabbit aortic internal elastic lamina and in the elastic lamellae of aortic media. *Microvascular Research*. 2007; 73(2):95–99. <https://doi.org/10.1016/j.mvr.2006.10.005>. [PubMed: 17174983]
- Fata B, Carruthers CA, Gibson G, Watkins SC, Gottlieb D, Mayer JE, Sacks MS. Regional structural and biomechanical alterations of the ovine main pulmonary artery during postnatal growth. *Journal of Biomechanical Engineering*. 2013; 135(2):21022. <https://doi.org/10.1115/1.4023389>.
- Fata B, Carruthers Ca, Gibson G, Watkins SC, Gottlieb D, Mayer JE, Sacks MS. Regional structural and biomechanical alterations of the ovine main pulmonary artery during postnatal growth. *Journal of Biomechanical Engineering*. 2013; 135(2):21022. <https://doi.org/10.1115/1.4023389>.
- Fung YC, Liu SQ. Change of residual strains in arteries due to hypertrophy caused by aortic constriction. *Circulation Research*. 1989; 65(5):1340–1349. [PubMed: 2805247]
- Gasser TC, Ogden RW, Holzapfel GA. Hyperelastic modelling of arterial layers with distributed collagen fibre orientations. *Journal of the Royal Society Interface*. 2006; 3(6):15–35. <https://doi.org/10.1098/rsif.2005.0073>.
- Glagov S, Zarins C, Giddens DP, Ku DN. Hemodynamics and atherosclerosis. Insights and perspectives gained from studies of human arteries. *Archives of Pathology & Laboratory Medicine*. 1988; 112(10):1018–1031. Retrieved from http://zarinslab.stanford.edu/publications/zarins_bib/zarins_pdf/1980s/glagov_aplm_aplm_1988.pdf. [PubMed: 3052352]
- Hill MR, Duan X, Gibson GA, Watkins S, Robertson AM. A theoretical and non-destructive experimental approach for direct inclusion of measured collagen orientation and recruitment into mechanical models of the artery wall. *Journal of Biomechanics*. 2012; 45(5):762–771. <https://doi.org/10.1016/j.jbiomech.2011.11.016>. [PubMed: 22305290]
- Hoffmeister BK, Handley SM, Wickline SA, Miller JG. Ultrasonic determination of the anisotropy of Young's modulus of fixed tendon and fixed myocardium. *The Journal of the Acoustical Society of America*. 1996; 100(6):3933–3940. <https://doi.org/10.1121/1.417246>. [PubMed: 8969490]
- Holzapfel GA, Gasser TC, Ogden RW. A New Constitutive Framework for Arterial Wall Mechanics and a Comparative Study of Material Models. *Journal of Elasticity*. 2000; 61(3):1–48.
- Holzapfel GA, Sommer G, Gasser CT, Regitnig P. Determination of layer-specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling. *American Journal of Physiology-Heart and Circulatory Physiology*. 2005; 289(5):H2048–H2058. [https://doi.org/10.1016/0021-9150\(85\)90008-5](https://doi.org/10.1016/0021-9150(85)90008-5). [PubMed: 16006541]
- Kao PH, Lammers SR, Tian L, Hunter K, Stenmark KR, Shandas R, Qi HJ. A microstructurally driven model for pulmonary artery tissue. *Journal of Biomechanical Engineering*. 2011; 133(5):51002. <https://doi.org/10.1115/1.4002698>.
- Ku DN. BLOOD FLOW IN ARTERIES. *Annual Review of Fluid Mechanics*. 1997; 29(1):399–434. Retrieved from <http://annualreviews.org/doi/pdf/10.1146/annurev.fluid.29.1.399>.
- Kuhn W, Grün F. Beziehungen zwischen elastischen Konstanten und Dehnungsdoppelbrechung hochelastischer Stoffe. *Colloid & Polymer Science*. 1942; 101(3):248–271. <https://doi.org/10.1007/BF01793684>.

- Lillie MA, Gosline JM. Mechanical properties of elastin along the thoracic aorta in the pig. *Journal of Biomechanics*. 2007; 40(10):2214–2221. <https://doi.org/10.1016/j.jbiomech.2006.10.025>. [PubMed: 17174959]
- Lillie MA, Shadwick RE, Gosline JM. Mechanical anisotropy of inflated elastic tissue from the pig aorta. *Journal of Biomechanics*. 2010; 43(11):2070–2078. <https://doi.org/10.1016/j.jbiomech.2010.04.014>. [PubMed: 20430395]
- Maiellaro-Rafferty K, Weiss D, Joseph G, Wan W, Gleason RL, Taylor WR. Catalase overexpression in aortic smooth muscle prevents pathological mechanical changes underlying abdominal aortic aneurysm formation. *American Journal of Physiology-Heart and Circulatory Physiology*. 2011; 301(2):H355–H362. <https://doi.org/10.1152/ajpheart.00040.2011>. [PubMed: 21551275]
- Ono K, Kikuch Y, Higashi K, Tamiya N, Yasuoka N. Elastic anisotropy of bivalve hinge-ligament. *Journal of Biomechanics*. 1990; 23(4):307311–309312. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/2335528>.
- Rezakhaniha R, Fonck E, Genoud C, Stergiopulos N. Role of elastin anisotropy in structural strain energy functions of arterial tissue. *Biomechanics and Modeling in Mechanobiology*. 2011; 10(4): 599–611. <https://doi.org/10.1007/s10237-010-0259-x>. [PubMed: 21058025]
- Rezakhaniha R, Stergiopulos N. A structural model of the venous wall considering elastin anisotropy. *Journal of Biomechanical Engineering*. 2008; 130(3):31017. <https://doi.org/10.1115/1.2907749>.
- Roach MR, Burton AC. The reason for the shape of the distensibility curves of arteries. *Biochemistry and Cell Biology*. 1957; 35(8):681–690. <https://doi.org/10.1139/o57-080>.
- Rodbard S. Negative feedback mechanisms in the architecture and function of the connective and cardiovascular tissues. *Perspectives in Biology and Medicine*. 1970; 13(4):507–527. <https://doi.org/10.1353/pbm.1970.0054>. [PubMed: 5474042]
- Ronchetti IP, Alessandrini A, Contri MB, Fornieri C, Mori G, Quaglino D, Valdre U. Study of elastic fiber organization by scanning force microscopy. *Matrix Biology*. 1998; 17(1):75–83. [https://doi.org/10.1016/S0945-053X\(98\)90126-3](https://doi.org/10.1016/S0945-053X(98)90126-3). [PubMed: 9628254]
- Sacks MS. Biaxial Mechanical Evaluation of Planar Biological Materials. *Journal of Elasticity*. 2000; 61(1):199. <https://doi.org/10.1023/A:1010917028671>.
- Sherebrin MH, Song SH, Roach MR. Mechanical anisotropy of purified elastin from the thoracic aorta of dog and sheep. *Canadian Journal of Physiology and Pharmacology*. 1983; 61(6):539–545. <https://doi.org/10.1139/y83-083>. [PubMed: 6883206]
- Timmins LH, Wu Q, Yeh AT, Moore JE, Greenwald SE. Structural inhomogeneity and fiber orientation in the inner arterial media. *American Journal of Physiology-Heart and Circulatory Physiology*. 2010; 298(5):H1537–H1545. <https://doi.org/10.1152/ajpheart.00891.2009>. [PubMed: 20173046]
- Wagenseil JE, Ciliberto CH, Knutsen RH, Levy MA, Kovacs A, Mecham RP. Reduced vessel elasticity alters cardiovascular structure and function in newborn mice. *Circulation Research*. 2009; 104(10): 1217–1224. <https://doi.org/10.1161/CIRCRESAHA.108.192054>. [PubMed: 19372465]
- Wan W, Dixon JB, Gleason RL. Constitutive modeling of mouse carotid arteries using experimentally measured microstructural parameters. *Biophysical Journal*. 2012; 102(12):2916–2925. <https://doi.org/10.1016/j.bpj.2012.04.035>. [PubMed: 22735542]
- Wang Y, Zeinali-Davarani S, Zhang Y. Arterial mechanics considering the structural and mechanical contributions of ECM constituents. *Journal of Biomechanics*. 2016; 49(12):2358–2365. <https://doi.org/10.1016/j.jbiomech.2016.02.027>. [PubMed: 26947034]
- Weiss JA, Maker BN, Govindjee S. Finite element implementation of incompressible, transversely isotropic hyperelasticity. *Computer Methods in Applied Mechanics and Engineering*. 1996; 135(1–2):107–128. [https://doi.org/10.1016/0045-7825\(96\)01035-3](https://doi.org/10.1016/0045-7825(96)01035-3).
- Wolinsky H, Glagov S. A lamellar unit of aortic medial structure and function in mammals. *Circulation Research*. 1967; 20(1):99–111. <https://doi.org/10.1161/01.RES.20.1.99>. [PubMed: 4959753]
- Wuyts FL, Vanhuyse VJ, Langewouters GJ, Decraemer WF, Raman ER, Buyle S. Elastic properties of human aortas in relation to age and atherosclerosis: a structural model. *Physics in Medicine and Biology*. 1995; 40(10):1577–1597. <https://doi.org/10.1088/0031-9155/40/10/002>. [PubMed: 8532741]

- Zeinali-Davarani S, Chow MJ, Turcotte R, Zhang Y. Characterization of biaxial mechanical behavior of porcine aorta under gradual elastin degradation. *Annals of Biomedical Engineering*. 2013; 41(7):1528. <https://doi.org/10.1007/s10439-012-0733-y>. [PubMed: 23297000]
- Zeinali-Davarani S, Wang Y, Chow MJ, Turcotte R, Zhang Y. Contribution of collagen fiber undulation to regional biomechanical properties along porcine thoracic aorta. *Journal of Biomechanical Engineering*. 2015; 137(5):51001. <https://doi.org/10.1115/1.4029637>.
- Zhang Y, Dunn ML, Drexler ES, McCowan CN, Slifka AJ, Ivy DD, Shandas R. A microstructural hyperelastic model of pulmonary arteries under normo- and hypertensive conditions. *Annals of Biomedical Engineering*. 2005; 33(8):1042–52. <https://doi.org/10.1007/s10439-005-5771-2>. [PubMed: 16133913]
- Zhang Y, Dunn ML, Hunter KS, Lanning C, Ivy DD, Claussen L, ... Shandas. Application of A Microstructural Constitutive Model of the Pulmonary Artery to Patient-Specific Studies: Validation and Effect of Orthotropy. *Journal of Biomechanical Engineering*. 2007; 129(2):193–201. <https://doi.org/10.1115/1.2485780>. [PubMed: 17408324]
- Zhou J, Fung YC. The degree of nonlinearity and anisotropy of blood vessel elasticity. *Proceedings of the National Academy of Sciences*. 1997; 94(26):14255–14260. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9405599>.
- Zou Y, Zhang Y. An experimental and theoretical study on the anisotropy of elastin network. *Annals of Biomedical Engineering*. 2009; 37(8):1572–1583. <https://doi.org/10.1007/s10439-009-9724-z>. [PubMed: 19484387]
- Zulliger MA, Fridez P, Hayashi K, Stergiopoulos N. A strain energy function for arteries accounting for wall composition and structure. *Journal of Biomechanics*. 2004; 37(7):989–1000. <https://doi.org/10.1016/j.jbiomech.2003.11.026>. [PubMed: 15165869]

Figure 1. Multiphoton images showing the circumferential cross-sectional view of elastin network. The images reveal transmural changes in the organization of elastin fibers in the outer, middle, and inner media.

Figure 2.

Multiphoton images showing the distribution of elastin fibers in the inner media (1), middle media (2, 3, 4), and outer media (5). Here L refers to longitude; and C refers to circumference.

Figure 3.

(a) Transmural variation in elastin fiber orientation distribution in the inner, middle, and outer media with 0° being the circumferential and 90° being the longitudinal direction. (b) Ratio of circumferentially to longitudinally distributed elastin fibers in the inner, middle, and outer media ($*p < 0.01$).

Figure 4.

Multiphoton images of elastin fibers in the inner media while the elastin network was subjected to equi-biaxial strain from 0% to 30% with an increment of 10%. Here L refers to longitude; and C refers to circumference.

Figure 5.

Three-dimensional plot showing elastin fiber orientation distribution in the inner media while the elastin network was subjected to biaxial strain of 0%C-0%L, 15%C-30%L, 30%C-15%L, and 30%C-30%L. The x- axis represents the fiber angle with 0° being the circumferential and 90° being the longitudinal direction; and the y- axis indicates the biaxial strain.

Figure 6.

Comparison of elastin fiber orientation distribution in purified elastin network and intact artery (Chow et al., 2014) in the unloaded condition with 0° and 90° being the circumferential and longitudinal direction, respectively.

Figure 7.

Cauchy stress vs. stretch in the circumferential (Circ) and longitudinal (Long) directions of sample 5 in Tables 1 and 2 when fitting three sets of biaxial testing data using (a) the proposed model in Equation (2) that considers transmural variation in elastin fiber orientation distribution, and (b) a model that considers only fiber orientation distribution in inner media in Equation (4). Symbols represent experimental measurements and lines represent modeling results.

Figure 8.

Cauchy stress vs. stretch in the circumferential (Circ) and longitudinal (Long) directions of sample 5 in Tables 1 and 2 when fitting two sets of nonequi-biaxial testing data $f_l:f_c = 75:100$ and $100:75$ N/m, and predicting equi-biaxial testing data $f_l:f_c = 100:100$ N/m using (a) the proposed model in Equation (2) that considers transmural variation in elastin fiber orientation distribution, and (b) a model that considers only fiber orientation distribution in inner media in Equation (2). Symbols represent experimental measurements and lines represent modeling results.

Summary of model parameters, n_{im} , n_{mm} and n_{om} , N , and fitting ($Error_f$) and predicting ($Error_p$) errors from the proposed model that considers transmural variation in elastin fiber orientation distribution. Parameters n_{im} , n_{mm} and n_{om} correspond to elastin content in inner, middle and outer media, respectively.

Table 1

Sample number	n_{im}	n_{mm}	n_{om}	N	$Error_f$	$Error_p$
Fitting equi- and nonequi- biaxial testing simultaneously						
1	1.42×10^{24}	2.84×10^{24}	1.42×10^{24}	1.4039	0.1453	-
2	5.38×10^{23}	9.68×10^{24}	5.38×10^{23}	1.9054	0.1125	-
3	1.45×10^{24}	3.85×10^{24}	5.16×10^{23}	1.5252	0.1189	-
4	8.88×10^{23}	1.60×10^{25}	8.88×10^{23}	1.8325	0.1273	
5	1.66×10^{24}	1.60×10^{25}	5.28×10^{23}	1.5047	0.0682	
6	6.17×10^{23}	1.11×10^{25}	6.17×10^{23}	1.7491	0.0899	
Mean	1.10×10^{24}	7.98×10^{24}	7.51×10^{23}	1.6535	0.1103	-
SEM	1.94×10^{23}	2.10×10^{24}	1.45×10^{23}	0.0828	0.0124	-
Fitting nonequi-biaxial testing and predicting equi-biaxial testing						
1	1.22×10^{24}	2.44×10^{24}	1.22×10^{24}	1.3712	0.1710	0.1266
2	5.64×10^{23}	1.01×10^{25}	5.64×10^{23}	1.9670	0.1170	0.1177
3	1.74×10^{24}	4.59×10^{24}	6.34×10^{23}	1.6054	0.1333	0.1154
4	7.72×10^{23}	1.39×10^{25}	7.72×10^{23}	1.7150	0.1204	0.1610
5	1.59×10^{24}	4.06×10^{24}	7.00×10^{23}	1.4865	0.0569	0.1126
6	4.75×10^{23}	8.54×10^{24}	4.74×10^{24}	1.5991	0.0736	0.1531
Mean	1.06×10^{24}	7.28×10^{24}	7.27×10^{23}	1.6240	0.1120	0.1311
SEM	2.19×10^{24}	1.78×10^{24}	1.07×10^{23}	0.0169	0.0169	0.0085

Table 2

Summary of model parameters, n and N , and fitting (Error_f) and predicting (Error_p) errors from model that only considers elastin fiber orientation distribution from inner media.

Sample number	n	N	Error_f	Error_p
Fitting equi- and nonequi- biaxial testing simultaneously				
1	6.21×10^{24}	1.4195	0.1667	-
2	1.34×10^{25}	2.9042	0.4636	-
3	1.21×10^{25}	1.7165	0.3614	-
4	7.74×10^{24}	1.5941	0.5233	-
5	1.25×10^{25}	1.9605	0.3394	-
6	5.46×10^{24}	1.5551	0.5099	-
Mean	8.84×10^{24}	1.8583	0.3940	-
SEM	1.36×10^{24}	0.2220	0.0550	-
Fitting nonequi-biaxial testing and predicting equi-biaxial testing				
1	6.14×10^{24}	1.4183	0.1830	0.1380
2	1.32×10^{25}	3.0953	0.4678	0.4570
3	1.21×10^{25}	2.0886	0.3676	0.3378
4	6.48×10^{24}	1.5152	0.5513	0.4977
5	6.03×10^{24}	1.5028	0.3445	0.4228
6	5.49×10^{24}	1.5234	0.5147	0.5574
Mean	8.24×10^{24}	1.8573	0.4048	0.4018
SD	1.41×10^{24}	0.2666	0.0553	0.0607