A validated 3D microstructure-based constitutive model of coronary artery adventitia

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ABSTRACT

A structure-based model that accurately predicts micro- or macro-mechanical behavior of blood vessels is necessary to understand vascular physiology. Based on recently measured microstructural data, we propose a 3-D microstructural model of coronary adventitia that incorporates the elastin and collagen distributions throughout the wall. The role of ground substance was found to be negligible under physiological axial stretch $\lambda = 1.3$, based on enzyme degradation of glycosaminoglycans in swine coronary adventitia ($n=5$). The thick collagen bundles of outer adventitia ($n=4$) were found to be undulated and unengaged at physiological loads, while the inner adventitia consisting of multiple sublayers of entangled fibers that bear the majority of load at higher pressures. The microstructural model was validated against biaxial (inflation and extension) experiments of coronary adventitia ($n=5$). The model accurately predicted the nonlinear responses of the adventitia, even at high axial force (axial stretch ratio $\lambda = 1.5$). The model also enabled a reliable estimation of material parameters of individual fibers that were physically reasonable. A sensitivity analysis was performed to assess the effect of using mean values of the distributions for fiber orientation and waviness as opposed to the full distributions. The simplified mean analysis affects the fiber stress-strain relation, resulting in incorrect estimation of mechanical parameters, which underscores the need for measurements of fiber distribution for a rigorous analysis of fiber mechanics. The validated structure-based model of coronary adventitia provides a deeper understanding of vascular mechanics in health and can be extended to disease conditions.
INTRODUCTION

Microstructural approaches have been advocated for modeling blood vessels to better understand the nonlinear mechanical responses of vessels (7, 17, 18, 25). Mechanical predictions are thought to be more accurate than those of phenomenological models as these models account for microstructural features of vessel components as well as heterogeneity of material properties. The microstructural parameters have been ad hoc rather than based on experimental measurements due to limited morphological data of vessel wall (4, 17, 23, 51). The microstructure of vessel wall which includes elastin and collagen fibers, smooth muscle cells and ground substance, are distributed differently in individual vessel layers, i.e., tunica media and adventitia (8, 36, 44). A microstructural constitutive model should be specific for a particular layer based on experimental measurements of microstructure (2).

The coronary adventitia consists of three major constituents: elastin, collagen fibers and ground substance, and the media is made up of concentric smooth muscle cell layers, elastic lamellae, and a few collagen and elastin fibers. At physiological pressures, the adventitia is less stiff than the media, while the latter serves as the most important mechanical layer to resist luminal pressure. At higher pressures (e.g., hypertension), collagen fibers reach their straightened lengths and the adventitia becomes a stiff tube to prevent the media from overstretch and rupture (3, 6, 43, 48). Smooth muscle cells in media predominate active responses of blood vessels, while elastin and collagen fibers only contribute to passive mechanical behaviors (5, 21). The mechanical properties of individual fibers have been broadly studied in literature (1, 13, 15, 20, 43, 46) and microstructural parameters and deformation in coronary adventitia have been quantitatively measured in our recent studies (3, 6). The mechanical function of ground substance, an amorphous gel-like material containing glycosaminoglycans (GAGs), proteoglycans, glycoproteins and fibroblasts, has yet to be tested, although some related studies have been performed for other type of tissues (22, 27, 31, 42, 43).

Two major classes of micromechanical models, with different assumptions for the matrix material (cells and ground substance) of the tissue, have been developed in the past decades. The first ones considered the vessel wall as a composite of elastin and collagen fibers embedded in a fluid-like matrix (17, 25, 5, 24, 45, 9). The fibers are the only constituent phases that sustain non-hydrostatic loading such as tension and shear, while the contribution of the fluid-like matrix is only a hydrostatic pressure. The second class of micromechanical models assume the tissue as a fiber reinforced composite, of which the non-collagenous matrix material (including elastin fibers, cells and ground substance) or the non-fibrous matrix (including cells and ground substance) is a
solid-like material that can take up load (18, 26, 50). One question is which of these two hypotheses (fluid-like or solid-like) is most representative of the adventitia ground substance?

A microstructural model should not only accurately predict the macro- and micro-mechanical behavior of blood vessels, but also provide a reliable approach for parameter identification of individual fibers. Tensile, micromechanical bending or nano-indentation tests done to determine the mechanical properties of individual fibers have shown significant variations of fiber stiffness (1, 13, 20, 46). Previous microstructural models employed simplified assumptions of fiber distribution (e.g., fibers aligning in two groups with symmetrical orientation angles) (17, 18, 50). Although these models provided good descriptions of nonlinear responses of the blood vessels, the predictive capabilities or parameter estimation were lacking. Hollander et al (17) employed a structure-based model that showed high descriptive and predictive capabilities for the nonlinear mechanical response of coronary media. The stiffness of elastin and collagen fibers was underestimated, however, due to lack of realistic microstructural parameters.

The goal of the present study was to validate a structure-based model of coronary artery adventitia based on microstructure of elastin, collagen and ground substance. To assess the role of ground substance on the mechanical response, adventitia specimens were mechanically tested, with and without GAGs under physiological axial stretch $\lambda_z$=1.3. Previous measurements of microstructural parameters were then incorporated into the proposed model to predict the nonlinear responses of adventitia and to estimate the material parameters. The model was validated based on biaxial (inflation and axial stretch) of swine coronary adventitia. The microstructural model accurately predicts the macro-mechanical biaxial vessel responses and provides reliable parameter estimations of individual fibers.

**MATERIALS AND METHODS**

**Sample Preparation**

Porcine hearts (n=14) were harvested at a local slaughterhouse and transported immediately to the laboratory in 4°C physiological salt solutions (PSS). The left anterior descending arteries were dissected away from their emergence at aortic ostia, and the adjacent tissue around the segments was dissected carefully. In ten hearts (n=10), the intima-media layer of an artery was carefully peeled off from the vessel wall to leave an intact adventitia segment. It is relatively easy to remove the intima-media layer without damage to the adventitia because there exists a cleavage plane at the external elastic lamellae that structurally separates the media and adventitia layers (36). Adventitia segments were then cut approximately 2 cm in length and kept in 4°C PSS.
**Distension-Extension Mechanical Testing**

The adventitia segments (n=5) prepared as described above were used for pressure distension-axial extension mechanical testing (30). The specimens were cannulated on both ends and connected in an organ bath containing PSS at room temperature. A pressure regulator was used to control luminal pressure, and a force transducer was connected to the right cannulate to measure axial force on the vessel. A video camera in conjunction with vessel diameter-tracking software was used to continuously track the outer vessel diameter during mechanical testing. Axial force, luminal pressure, and outer diameter were recorded by a data acquisition system (MP 100, Biopac Systems, CA). The segment was elongated to 3 different axial stretch ratios: \( \lambda_x = 1.0, 1.3 \) and 1.5, respectively. While the segment was gradually inflated at a fixed axial stretch, the diameter-pressure and axial force-pressure curves were measured. Immediately after measurements, the segment was taken out and a 2 mm-long ring was cut. The ring was then cut open radially to release the residual stresses and strains (12); i.e., zero-stress state (ZSS) of a specimen, of which the outer and inner radii and opening angles were measured. All specimens were preconditioned before mechanical testing to obtain reproducible data (12).

**Histology of Pressurized Coronary Arteries**

In additional hearts (n=4), the intact vessel segment was carefully dissected. For each heart, three segments of ~2 cm in length were prepared for different distension pressures: 0 mmHg (no-load state), 100 mmHg, and 200 mmHg, respectively. A custom-made excess surface-area balloon tip catheter was inserted into vessel segment and distended to fully transmit the pressure to the vessel lumen. The excess surface area of balloon ensured that no pressure was taken up by the balloon itself (48). The balloon-distended segment was immersion-fixed in 0.8% methanol-free paraformaldehyde solution at room temperature for 48 hrs. (5). A ~5 mm ring was then cut from the fixed segment, embedded in OCT compound and instantly frozen by liquid nitrogen. The cross sections of frozen segment were sectioned by a cryostat microtome, mounted on microscope slides and viewed by an FV1000-MPE multiphoton microscope (MPM) (Olympus America, Center Valley, PA), which is equipped with a Spectra-Physics MaiTai DeepSee tunable laser. A combination of second harmonic generation (SHG) and two-photon excitation fluorescence (TPEF) was set up to simultaneously detect signals of collagen and elastin fibers. The excitation wavelength of the laser was 830 nm, and emission wavelengths for collagen (SHG) and elastin (TPEF) were 415 nm and 520 nm, respectively. MPM images of cross sections of distended vessels were collected with 512 x 512 pixel² resolution. Another 5x5mm² lateral section (as
shown in Fig. 1d) was cut from the unloaded vessel segment and imaged by MPM to visualize fiber arrangement on lateral surface of arterial adventitia.

**Removal of Glycosaminoglycans in Coronary Adventitia**

In addition to the elastin and collagen fibers in the adventitia, there are also GAGs, structural glycoproteins, plasma proteins and fibroblasts, which are collectively constituents of the ground substance (an amorphous gel-like media). Since there are few fibroblasts in coronary adventitia and their passive mechanical responses are weak (10), GAGs and their associated proteins (i.e., proteoglycans and glycoproteins) are considered as the major components of ground substance.

To determine the mechanical contribution of the ground substance, GAGs can be digested by Chondroitinase ABC (Sigma-Aldrich, MO) (11, 22, 42). Intact arterial specimens (the segments of ~5 mm in length were cut from the hearts (n=4) used for the histology study) were used to confirm digestion of GAGs since adventitia specimens were too thin to be sectioned in later confirmatory immunohistochemical studies. A 0.2 U/ml solution of Chondroitinase ABC was used to incubate the vessels in 37°C water bath with shaker for 1.5 hrs. Since the Anti-Chondroitin Sulfate antibody, CS-56, recognizes an epitope on intact chondroitin sulfate GAGs chains (19, 47), GAGs were labeled with CS-56 (Abcam, Cambridge, MA). After vessel specimens were incubated with the CS-56-specific mouse monoclonal antibody, they were visible with an ABC staining system (Santa Cruz Biotechnology, CA) and examined by light microscopy. Controls were obtained by using arterial segment incubated without the CS-56 primary antibody.

After confirming removal of GAGs in intact specimens, a separate set of adventitia segments (n=5) were treated with Chondroitinase ABC. Mechanical testing was performed before and after GAGs removal. The segment was stretched to \( \lambda_z = 1.3 \) (approximating in vivo length) and inflated by different pressures to obtain the diameter-pressure curve of arterial adventitia with or without GAGs.

**A 3D Microstructure-based Model of Coronary Adventitia**

The adventitia was considered as a cylindrical tube, with the following kinematic assumptions: 1) the adventitia is incompressible; 2) deformations are axis-symmetric and independent of axial position; 3) transverse sections remain planar; and 4) there is a unique undeformed reference configuration (i.e., ZSS). We used a cylindrical coordinate system with circumferential direction \( g_1 \), radial direction \( g_2 \) and axial direction \( g_3 \) as principal directions, the corresponding stretches \( \lambda_\theta, \lambda_r \) and \( \lambda_z \) were determined, respectively,

\[
\lambda_\theta = \left( \frac{\pi}{\pi - \theta_0} \right) \frac{r}{R}, \quad \lambda_r = \frac{\partial r}{\partial R}, \quad \lambda_z = \frac{l}{L}
\]  

(1)
where $\theta_0$ is opening angle measured at ZSS, $R$ is radius to a point at ZZS and $r$ is the radius to the same point in the current configuration. $L$ is the axial length of the segment at ZSS and $l$ is the loaded axial length. According to material incompressibility: $J = \lambda_\theta \lambda_r \lambda_z = 1$, for the mapping between ZSS and loaded state, loaded radius $r$ was determined as a function of unloaded $R$:

$$r(R) = \sqrt{r_o^2 - (R_o^2 - R^2) \frac{\pi - \theta_0}{\lambda_z \pi}}$$

(2)

where $r_o$ is the outer radius in the loaded state while $R_o$ is that at ZSS.

The radial component of the force equilibrium equation imposed on the loaded configuration is given by:

$$\frac{\partial \sigma_{rr}}{\partial r} + \frac{\sigma_{rr} - \sigma_{\theta \theta}}{r} = 0$$

(3)

where $\sigma_{ij}$ as Cauchy stress tensor. According to boundary conditions $\sigma_{rr}|_{r_l} = -p_l$, $\sigma_{rr}|_{r_o} = 0$,

the luminal pressure $p_l$ can be written as:

$$p_l = \int_{r_l}^{r_o} \frac{(\sigma_{\theta \theta} - \sigma_{rr})}{r} dr$$

(4)

The axial force that was required to maintain the vessel axial stretch was given by:

$$F = \pi \int_{r_l}^{r_o} (2\sigma_{zz} - \sigma_{\theta \theta} - \sigma_{rr}) r dr$$

(5)

**Strain-Energy Function**

The coronary adventitia was considered as an incompressible hyper-elastic solid and characterized by a strain energy function $W(E)$ as a function of the Green-Lagrange strain tensor $E = \frac{1}{2} (F^T \cdot F - I)$. The Cauchy stress tensor $\sigma$ is given by (16)

$$\sigma = F \frac{\partial W}{\partial E} F^T - pI = F \cdot S \cdot F^T - pI$$

(6)

where $F$ is the deformation gradient tensor and $S$ is the second Piola-Kirchhoff stress tensor. $I$ is the second order identity tensor, scalar $p$ is hydrostatic pressure, which acts as a Lagrange multiplier and must be determined from equilibrium and boundary conditions.

The strain energy function (SEF) $W(E)$ of a microstructural model involves structural features. Our experimental studies showed that coronary adventitia is divided into outer and inner adventitia as shown in Fig.1 (13,14). The outer adventitia, consisting of thicker and wavier collagen bundles and few elastin fibers, supports the vessel and connects with the surrounding tissue rather than significantly resist the transmural pressure (Fig.1e, f), while the inner adventitia is a layered structure with concentric densely packed fiber sheets and has few radial fiber bundles.
distributing between sheets. At low pressures, elastin fibers bear the loads and collagen fibers are still wavy in inner adventitia. At high pressures, stretched collagen fibers in inner adventitia are recruited to withstand stresses. Therefore, the adventitia wall was modeled as a composite containing two mechanical components: collagen and elastin fibers, while ground substance was shown to have a negligible mechanical function (Fig. 2) and treated as a fluid that sustains hydrostatic pressure. Both types of fibers are only resistant to tensile load, undulated collagen fibers are recruited to bear loads only after they become straightened, and there is no interaction between collagen and elastin fibers.

A fluid-like matrix implies the tissue undergoes affine deformations; i.e., deformation of the fibers is the same as that of ground substance. Based on this assumption, the SEF of adventitia wall can be represented by the volume-weighted summation of individual SEF of elastin fiber \( W_E \) and collagen fiber \( W_C \) (25):

\[
W(E) = f_E W_E + f_C W_C 
\] (7)

where \( f_i \) \((i = E, C)\) is the volume fraction of each type of fiber \( i \). Generally, the orientation of fibers follows a continuous distribution density function, and the volume weighted SEF of fibers is given by (25):

\[
W_i = \int_0^\pi \mathcal{R}_i(\theta)w_i(e) \, d\theta 
\] (8)

where \( w_i(e) \) is the SEF of individual fibers as a function of fiber strain \( e \). It should be noted that radially oriented elastin and collagen fibers are not engaged under inflation-extension condition where they are compressed and bear no loads, and only planar fibers contribute to mechanical behavior of the adventitia. Thus, \( \mathcal{R}_i(\theta) \) is a planar orientation distribution density function of fiber \( i \), and \( \theta \) is the angle between the fiber orientation and the circumferential direction of the vessel \( g_1 \). \( \mathcal{R}_i(\theta) \) satisfies the normalization criterion \( \int_0^\pi \mathcal{R}_i(\theta) \, d\theta = 1 \). The uniaxial fiber strain \( e(\theta) \) is determined by the local strain tensor \( E \) and the reference fiber direction \( N = \{ \cos \theta, \sin \theta \} \) as:

\[
e(E, N) = E : N \otimes N
\] (9)

Although elastin and collagen fibers distributed in each sublayer with transmural variation of fiber orientation, a mixture of two normal distribution of fiber orientation through adventitia wall was found to describe the data as (6):

\[
W_i = \left\{ \omega_{i1} \int_0^\pi \mathcal{R}_{i1}(\theta)w_i(e) \, d\theta + \omega_{i2} \int_0^\pi \mathcal{R}_{i2}(\theta)w_i(e) \, d\theta \right\} 
\] (10)
where $R_{ij}(\theta) = \frac{1}{K_j \sigma_j / 2\pi} \exp \left[-\frac{(\theta - \mu_j)^2}{2\sigma_j^2}\right]$, $(i = E, C; j = 1, 2)$ is a truncated normal density function with $\mu_j$ and $\sigma_j$ as the mean and standard deviation, respectively. $K_j$ is a truncated parameter $K_j = \Phi\left(\frac{\pi - \mu_j}{\sigma_j}\right) - \Phi\left(\frac{-\mu_j}{\sigma_j}\right)$ ($\Phi$ is the cumulative distribution function of a normal distribution), and $\omega_{ij}$ is the weight of each normal distribution ($\sum_{j=1}^{2} \omega_{ij} = 1$). The second Piola-Kirchhoff stress of each type of fiber was derived as:

$$S_i = \sum_{j} \omega_{ij} \int_{0}^{\pi} R_{ij}(\theta) \frac{\partial w_i}{\partial e} d\theta$$

(11)

According to microscopic responses of individual elastin fibers under mechanical loads (3, 6), the elastic properties was assumed to be linear:

$$\frac{\partial w_E}{\partial e} = \begin{cases} 0 & e < 0 \\ k_E e & e > 0 \end{cases}$$

(12)

where $k_E$ is stiffness parameter of elastin fiber. Because of the wavy nature of collagen fibers, the nonlinear constitutive relation was considered to account for the nonlinear elastic behavior (17):

$$\frac{\partial w_C}{\partial e} = \begin{cases} 0 & e \leq e_0 \\ k_c (e - e_0)^{M_c} & e > e_0 \end{cases}$$

(13)

where $k_c$ and $M_c$ are parameters characterizing the nonlinear stress-strain response of collagen, and $e_0$ denotes the strain beyond which the collagen can withstand tension, which was found to follow a Beta distribution for the coronary adventitia (6):

$$D(e_0) = \frac{1}{B(\alpha_1, \alpha_2)} \frac{(e_0 - a)^{\alpha_1-1} (b - e_0)^{\alpha_2-1}}{(b - a)^{\alpha_1+\alpha_2-2}}$$

(14)

where $B(\alpha_1, \alpha_2)$ is a Beta function, and $a$ and $b$ the lower and upper bounds of the straightening strain $e_0$. The constitutive law of collagen fiber thus can be written as:

$$\frac{\partial w_C}{\partial e} = \begin{cases} 0 & e \leq e_0 \\ k_c \int_{a}^{b} D(e_0) (e - e_0)^{M_c} d\theta & e > e_0 \end{cases}$$

(15)

Although the microstructural approach can employ any well-defined constitutive model for the fibers, we employed a linear function (Eq. 12) and a power function (Eq. 13) for elastin and collagen fibers, respectively, following our previous study (17). If we substitute the constitutive laws for individual elastin and collagen fibers (Eqs. 12-15) into Eqs. 6 and 11, the Cauchy stress components of the vessel were obtained. Given the geometrical parameters ($f_E, f_C$) and distribution functions ($R_{ij}(\theta), \omega_{ij}, D(e_0)$) measured in our recent study (summarized in Table 1) (6), there were only three unknown material parameters: $k_E, k_c$ and $M_c$ that need be
determined by the boundary condition (Eqs. 4 and 5). The integrals of the above equations do not have analytical expressions, so numerical approaches were employed.

Parameter Estimation

Parameters were optimized by least squares fit to the experimental data by minimizing an objective function based on the sum of squared residuals (SSE) between model predictions and experimental data. The objective function was defined as follows:

\[
SSE = \frac{1}{2} \frac{1}{nm} \sum_{i,j} \left[ \frac{(r_{ij} - \bar{r}_{ij})^2}{\sigma_{r}} + \frac{(F_{ij} - \bar{F}_{ij})^2}{\sigma_{F}} \right]
\]

where \(i\) and \(j\) denote distension and axial loads at which the corresponding outer radius \(r_{ij}\) and axial force \(F_{ij}\) were measured, \(n\) is the number of different pressures and \(m\) the number of different axial stretch ratios used. \(\sigma\) is the standard deviation of experimental measurement, and \(\bar{r}_{ij}, \bar{F}_{ij}\) are corresponding model predicted outer radius and axial force, which were determined by numerically solving (4) and (5). A numerical nonlinear optimization function \texttt{NMinimize} in Mathematica (WOLFRAM, US) was used to find a global minimum of the objective function (Eq. 16) and to determine three unknown parameters \((k_E, k_C, M_C)\) with the constraints: \((k_E > 0, k_C > 0, M_C > 0)\).

RESULTS

MPM images (Fig. 1a, b) showed that the outer adventitia consists of few elastin fibers and abundant collagen bundles which are much thicker and wavier than those of inner adventitia. With increase of distension pressure, both elastin and collagen fibers of inner adventitia were stretched to bear the loads (3, 6), while collagen bundles of outer adventitia were still wavy (Fig. 1c,e-f). Although image processing based on cross-section images of the 3D structure cannot accurately characterize the fiber structure of outer adventitia, qualitative analysis confirmed that collagen bundles are still undulated even under high pressures. This suggests that the major function of outer adventitia is to connect with the surrounding tissue rather than to bear the distension pressure. Removal of GAGs in adventitia was confirmed by immunohistochemical images as shown in Fig. 2 a-c. Intact arterial samples were stained with an ABC kit without CS-56 antibody incubation (Fig. 2a, control group, purple), and some samples were labeled with...
antibody CS-56 to specify GAGs followed by ABC staining (Fig. 2b, brown is specific for GAGs). GAGs-digested samples labeled with antibody CS-56 showed purple color indicating that few GAGs were left in adventitia after digestion (Fig. 2c). Figure 2d provides the pressure-diameter curves of adventitia segments before and after treatment of Chondroitinase ABC under physiological axial stretch $\lambda_z = 1.3$. The data did not show a significant difference between these two groups ($P=0.384$) which suggests the mechanical contribution of ground substance can be ignored at this load range.

The least squares estimation of 3 material parameters of fibers ($k_E, k_C, M_C$) is summarized in Table 2. The mean stiffness parameter of elastin fiber was $k_E=192.2\pm72.6$ kPa, while the parameters of collagen fiber were $k_C=27.2\pm5.1$ MPa and $M_C=5.37\pm0.53$. The parameter estimation for sample mean (average over 5 experimental data sets) is also given. The measured outer radius-pressure and axial force-pressure relations are compared with model predictions as shown in Fig. 3, under three different axial stretch ratios $\lambda_z = 1.0, 1.3$ and 1.5, respectively. The predictions are in good agreement with experimental measurements which accurately capture the nonlinear responses of coronary adventitia. The transmural stress distributions for the three Cauchy components: radial stress $\sigma_{rr}$, circumferential stress $\sigma_{\theta\theta}$ and axial stress $\sigma_{zz}$, are shown in Fig. 4 (top row). There are negligible differences between radial stresses $\sigma_{rr}$ at the three different stretch ratios, while the differences for circumferential stresses $\sigma_{\theta\theta}$ and axial stresses $\sigma_{zz}$ are significant at different stretch ratios. The circumferential stress $\sigma_{\theta\theta}$ at $\lambda_z = 1.0$ is lower than that of $\lambda_z = 1.3$ and 1.5, while the axial stress $\sigma_{zz}$ was close to zero at $\lambda_z = 1.0$ and rapidly increased at $\lambda_z = 1.5$. This is consistent with the change of stress components as a function of circumferential strain (Fig.4, bottom row).

The material parameters were also estimated from the data under two axial stretches and then used to simulate responses under the third axial stretch ratio. Model predictive power was examined by comparing model predictions (in terms of SSE) based on partial and full data sets. Parameter estimates based on partial database of axial stretch ratios of $\lambda_z = 1.3$ and 1.5 are summarized in Table 3. The mean stiffness of elastin was $k_E=139.5\pm73.0$ kPa and the parameters of collagen were $k_C=57.8\pm11.7$ MPa and $M_C=5.92\pm0.43$, with an increased total SSE ($0.68\pm0.15$). Material parameter estimation based on partial database of axial stretch ratios of $\lambda_z = 1.0$ and 1.3 were also performed, but showed a lower total SSE.

A sensitivity analysis was performed to replace the continuous spatial distributions of fiber orientation and waviness with the mean values. We considered two groups of elastin with two mean orientation angles ($\mu_{E1}=0.33$ and $\mu_{E2}=1.99$), and two groups of collagen with mean orientation angles ($\mu_{C1}=0.37$ and $\mu_{C2}=1.91$) with a unique straightening strain $e_0 = 0.345$. 
Parameter estimates of individual fibers were shown in Table 4. The stiffness parameter of elastin was \( k_E = 183.5 \pm 36.5 \) kPa, which was similar with \( k_E \) estimated by the model with full fiber distributions. The mean material parameters of collagen fibers were \( k_C = 51.6 \pm 14.9 \) MPa and \( M_C = 4.58 \pm 0.2 \), which were somewhat different with that of full distributions. The simplified approach achieved a slightly increased mean value of SSE as compared with that of full distributions (0.45\( \pm \)0.05 vs. 0.42\( \pm \)0.03).

Figure 5 shows the stress-strain curves of individual fibers using material parameters based on full fiber distributions with all experimental data sets (E1, C1), full distributions with partial data sets (E2, C2), and simplified mean values with full data sets (E3, C3), respectively. The stiffness of elastin was small compared with that of collagen fibers, and collagen showed a strong nonlinear response. The stress-strain curves of collagen fibers based on full distributions (C1 and C2) were close to each other with similar toe regions, while the one estimated by the simplified mean values (C3) showed a steeper stress-strain slope. The stress-strain curves C1 and C2 were comparable to the circumferential Cauchy stress-strain curve of the adventitia at middle wall \( \sigma_{\theta\theta} \).

**DISCUSSION**

We have recently provided a comprehensive study of vessel wall microstructure including measurement of geometries of elastin and collagen fibers (3), along with their microstructural deformation (3, 6) to integrate the data into microstructural model of coronary media (5). Here, we validated a 3D microstructural constitutive model for the coronary adventitia based on measured microstructural features. The role of ground substance in coronary adventitia was shown to be negligible and the major function of outer adventitia is to connect with the surrounding tissue rather than to bear loads at higher pressures as seems to be the function of the inner adventitia. The inner adventitia is hence the major mechanical layer of adventitia, of which the geometrical parameters and distributions of fibers have been quantitatively measured (3, 6). The proposed model is deep-rooted in micro-structure of the adventitia to ensure high descriptive and predictive capabilities and to provide reliable parameter estimations.

The present study experimentally confirmed that ground substance plays a negligible role in mechanical support of coronary adventitia under tension and hence can be considered as a fluid-like matrix. GAGs are the major components to resist compression, however, since the hyaluronate molecules of GAGs take up a hydrostatic volume 1000 times the space occupied by the molecules. Hence, GAGs resist vessel wall compression to prevent luminal radius collapse
from smooth muscle cell contraction (42). Collagen and elastin fibers embedded in fluid-like matrix satisfy an affine deformation field as a model of coronary adventitia. The inner adventitia is a layered structure with concentric densely packed fiber sheets with few radially-oriented fibers within the inner adventitia (3). Our previous measurements showed that ~80% of collagen fibers oriented towards the longitudinal direction (with \( \mu_{C2} = 1.91, \sigma_{C2} = 0.50, w_{C2} = 0.78 \)) (Table 1) and the other fibers aligned nearly in the circumferential direction (with \( \mu_{C1} = 0.37, \sigma_{C1} = 0.20, w_{C1} = 0.22 \)), following two normal distributions. This model differs than the assumption used in most microstructural models where fibers are symmetrically disposed with respect to the circumferential direction of the vessel (with a preferred orientation) (17, 18, 50). The longitudinal arrangement of fibers accounts for the significant increase of axial force at high stretch ratio \( \lambda_z = 1.5 \) (Fig. 3, bottom row). The other geometrical parameters, including fiber waviness distribution and volume fraction, were also employed to achieve a realistic microstructure-based model of coronary adventitia.

The proposed model accurately predicted the nonlinear responses of adventitia as shown in Fig. 3. The outer radius of adventitia rapidly increased at low pressure and began to plateau at higher pressures as a result of circumferentially-oriented collagen fibers engaged to withstand loads with increase of pressure. The outer radius slightly declined at elevated axial stretch ratio, while the axial force was found to increase greatly at \( \lambda_z = 1.5 \). The axial force was very low under \( \lambda_z = 1.0 \) where only elastin fibers contributed and collagen fibers were undulated and unengaged. When \( \lambda_z = 1.3 \) (equivalent strain was 0.345), a few longitudinal-oriented collagen fibers were recruited so that axial force increased moderately. When \( \lambda_z \) reached 1.5, which was beyond straightening strains of most collagen fibers, most longitudinal-oriented fibers were recruited to provoke a rapid increase of axial force and axial stress (as shown in bottom panel of Fig. 3 and right panel of Fig. 4). The distributions for Cauchy stress components were plotted in Fig. 4. The radial stresses were very low as the vessel wall (i.e., radial direction) was compressed under distension-extension protocol so that radial-oriented fibers would not provide support even if they existed. As axial stretch load increased, more and more longitudinal-oriented collagen fibers were engaged to take up loads, leading to elevated circumferential and longitudinal stresses, in line with change of outer radius and axial force.

It should be noted that the axial stretch of coronary arteries during the cardiac cycle may be about 10% smaller or larger than the physiological axial stretch ratio of 1.3 (33), which is included in the range of mechanical testing protocol: \( \lambda_z = 1.0, 1.3 \) and 1.5. In systole, collagen fibers are likely unengaged and the adventitia can shrink axially, while fibers become straightened to withstand tension such that the adventitia becomes stiffer against axial overstretch.
in diastole. The natural undulation of collagen fibers provides the coronary arteries less resistance to axial compression in systole, while their axial alignment prevents coronary arteries from overstretching in diastole. The structure-function relation of the collagen orientation in coronary arteries is in contrast with the predominantly circumferential alignment of collagen in other types of arteries that do not undergo axial dynamic deformation (14, 37–39).

Model predictive power was determined in terms of SSE by comparing predictions based on partial and full data sets. The results show the model reliably predicts the response of experimental data ($\lambda_z = 1.0$) based on material parameters estimated by model calibration ($\lambda_z = 1.3$ and 1.5). Estimation based on database of $\lambda_z = 1.0$ and 1.3, however, provided a poor SSE for the response at $\lambda_z = 1.5$. This was caused by adventitia fibers largely oriented in longitudinal arrangement, and most collagen fibers were still wavy and unengaged at low axial stretch ratio. These findings suggest that calibration of model based on database under large loads may be more accurate (SSE=0.34±0.08 for partial database of $\lambda_z = 1.3$ and 1.5 as shown in Table 3). The stiffness parameter of elastin $k_E$ was estimated at magnitude of 102 kPa, which is within the reported values of Young’s modulus: 100 kPa to 1 MPa (15). The elasticity modulus of collagen fiber has a large reported range of 100 to 1000 MPa, depending on testing methodology, specimen dimension, animal species, etc. (1, 13, 20, 46). The estimated value of parameter $k_C$ indicates that the tangent modulus (also called as Young’s modulus) of collagen fibers has a magnitude of about 10 MPa. Based on the estimated nonlinear stress-strain curves (C1 and C2 in Fig. 5) of single collagen fiber, the tangent moduli, determined at fiber strain $e_f = 1.0$ (fiber stretching 30% after becoming straightened), were 23.0 and 42.7 MPa for C1 and C2, respectively. The stress of C1 begins to rise at a steep slope at a critical strain ($e_f \approx 0.7$), while the stress of C2 rapidly increased at a similar critical strain but with a steeper slope, indicating that estimates based on database of larger axial stretch ratios $\lambda_z = 1.3$ and 1.5 provide a higher fiber stiffness that is closer to the reported values. The curve C3 determined by the simplified mean values model has a smaller critical strain that was largely induced by the unique straightening strain of collagen fibers ($e_0 = 0.345$). There were no collagen fibers engaged to withstand loads before fiber strain reached 0.345, so the material response of individual fiber have to match the overall mechanical response of adventitia wall, resulting in an overestimation of $k_C$ and underestimation of $M_C$ (as shown in Table 2 and 4). This suggests that the estimated parameters are not independent of structure if the model has microstructural insufficiency.

A recently developed microstructural model of coronary media included comprehensive microstructural features of coronary media (qualitatively) and provided good predictions of media twist response based on parameters estimated from only biaxial tests of inflation and extension.
The general model contained a total of 12 material and microstructural parameters. The estimated $k_E$ and $k_C$, however, were very low. The elastin stiffness $k_E$ had a magnitude of 1 kPa and was ignored in a reduced model (which only contained 4 parameters) (17). It was thought that since the helical elastin fibers are aligned in parallel to the helical collagen, the model cannot provide reliable estimate of the parameter based on the physiological loading data. The material parameter $k_C$ of collagen fiber was also underestimated as 400 kPa, along with a model estimated fiber orientation distribution and zero strengthening strain (i.e., $e_0 = 0$). Although the model showed high predictive power, it was not able to provide reliable parameter estimates for the media.

The adventitia shows stiffer longitudinal response with preferred axial collagen arrangement, while the media is stiffer in the circumferential direction (34), forming a heterogeneous and anisotropic intact coronary artery. The media consists of concentric smooth muscle cell layers, elastin lamellae, collagen fibril bundles and a network of elastic fibrils, serving as the most important mechanical layer at physiological conditions. Chen et al. recently developed a microstructural model including measured microstructural data and active properties of smooth muscle cells to accurately predict biaxial vasoactivity of coronary artery media (5). A future study that integrates the media with the adventitia model is needed to provide a two-layer microstructural model of an intact coronary artery. In addition, several multi-scale models have been developed to account for nanoscale effects, which account for interactions between cellular actin filaments and intra-cellular elastin and collagen fibrils (44–46). Although these models may shed light on the micromechanical environment of vessel wall, their application and development are hindered by lack of accurate mechanical and geometrical properties of constituents at the nanoscale. It is expected that further advancements in the state-of-the-art high-resolution noninvasive imaging techniques as well as 3D image processing algorithms will advance the development of multi-scale microstructural models.

There are several limitations in the present study. First, since geometrical parameters (orientation, diameter and waviness) of individual collagen fibers in outer adventitia cannot be obtained based on current 2D images of cross sections, 3D segmental algorithms should be developed to render the 3D fiber morphology and to extract accurate microstructural parameters (28, 29). Second, a previous study showed that there exists a correlation between fibril width and fiber strength (35) and large collagen bundles in outer adventitia may or may not have different stiffness compared to inner adventitia fibers. Experimental studies on collagen fibrils of vascular tissues, however, are needed to clarify the potential differences between the collagen fibers in the inner and outer adventitia. Third, collagen fibers in outer adventitia may engage at higher loads...
(over 200 mmHg) and can be accounted for in the current model. Fourth, an extended loading range (i.e., $1.0 \leq \lambda_z \leq 1.3$) should be considered for testing the adventitia with or without GAGs to confirm GAGs negligible effects on adventitia stiffness in a full strain range. Finally, although two-dimensional protocols of distension and extension are sufficient to provide reliable estimation of mechanical response (17), three-dimensional protocols (with torsion) should be considered for inclusion of shear, and additional data are necessary to obtain more rigorous estimates of material parameters of individual elastin and collagen fiber.

CONCLUSIONS

The proposed 3D microstructural model includes realistic microstructural features to provide an accurate and reliable prediction of the mechanical properties of the coronary artery adventitia. It also enabled reliable parameter estimations of individual elastin and collagen fibers. The present model can be applied to normal vessels as in the current study and it can be extended to disease vessels in the future when the altered microstructural features are documented to improve our understanding of initiation and progression of cardiovascular disease.

ACKNOWLEDGEMENTS

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REFERENCE


<table>
<thead>
<tr>
<th>Geometries</th>
<th>Fiber type</th>
<th>Distribution type</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation angle</td>
<td>Collagen</td>
<td>Bimodal normal distribution</td>
<td>$\mu_C^1=0.37, \sigma_C^1=0.20, w_C^1=0.22$</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$\mu_C^2=1.91, \sigma_C^2=0.50, w_C^2=1.0-w_C^1$</td>
</tr>
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<td></td>
<td>Elastin</td>
<td>Bimodal normal distribution</td>
<td>$\mu_E^1=0.33, \sigma_E^1=0.20, w_E^1=0.4$</td>
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<td></td>
<td></td>
<td></td>
<td>$\mu_E^2=1.99, \sigma_E^2=0.57, w_E^2=1.0-w_E^1$</td>
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<tr>
<td>Waviness</td>
<td>Collagen</td>
<td>Beta distribution</td>
<td>$a_1=5.01, a_2=52.67, a=0.0, b=4.0$</td>
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<tr>
<td>Volume fraction</td>
<td>Collagen</td>
<td></td>
<td>$f_C=33%$</td>
</tr>
<tr>
<td></td>
<td>Elastin</td>
<td></td>
<td>$f_E=22%$</td>
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</table>

A recent study found that the minor orientation of elastin was approximately orthometric to the major orientation in each sublayer of adventitia, and some elastin fibers in the minor direction were covered by fibers in the major direction at no-load or low-load states (Fig. 5 in Ref. (6)), suggesting $w_{E1}$ was larger than the value (0.19) measured at ZSS.
Table 2

Parameter estimates of individual elastin and collagen fibers based on full distension-extension experimental data. (SEM denotes standard error of the mean)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample No.</th>
<th>Average±SEM</th>
<th>Sample mean</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>$k_E$ (kPa)</td>
<td>29.37</td>
<td>20.01</td>
<td>171.2</td>
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<tr>
<td>$K_C$ (MPa)</td>
<td>45.8</td>
<td>19.8</td>
<td>15.3</td>
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<tr>
<td>$M_C$</td>
<td>5.22</td>
<td>4.88</td>
<td>4.00</td>
</tr>
<tr>
<td>SSE for $r_o$</td>
<td>0.19</td>
<td>0.22</td>
<td>0.31</td>
</tr>
<tr>
<td>SSE for $F$</td>
<td>0.30</td>
<td>0.29</td>
<td>0.07</td>
</tr>
<tr>
<td>Total SSE</td>
<td>0.50</td>
<td>0.51</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Table 3

Parameter estimates of individual elastin and collagen fibers based on experimental data of \( \lambda_z = 1.3 \) and 1.5

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample No.</th>
<th>Average±SEM</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>( k_E ) (kPa)</td>
<td>35.7</td>
<td>15.3</td>
</tr>
<tr>
<td>( K_C ) (MPa)</td>
<td>90.5</td>
<td>22.5</td>
</tr>
<tr>
<td>( M_C )</td>
<td>6.51</td>
<td>5.08</td>
</tr>
<tr>
<td>SSE for partial database of ( \lambda_z = 1.3 ) and 1.5</td>
<td>0.17</td>
<td>0.28</td>
</tr>
<tr>
<td>SSE for full database</td>
<td>0.29</td>
<td>0.53</td>
</tr>
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Table 4
Parameter estimates of individual elastin and collagen fibers based on a simplified model in comparison with the full continuous distributions of fiber orientation and waviness

<table>
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<tr>
<th>Parameters</th>
<th>Sample No.</th>
<th>Average±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>$k_E$ (kPa)</td>
<td>153.2</td>
<td>37.7</td>
</tr>
<tr>
<td>$K_C$ (MPa)</td>
<td>106.8</td>
<td>20.3</td>
</tr>
<tr>
<td>$M_C$</td>
<td>4.97</td>
<td>4.08</td>
</tr>
<tr>
<td>SSE for $r_o$</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td>SSE for $F$</td>
<td>0.38</td>
<td>0.25</td>
</tr>
<tr>
<td>Total SSE</td>
<td>0.58</td>
<td>0.39</td>
</tr>
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</table>
Figure Captions

Figure 1. (a) Outer adventitia (OA) consists of thicker collagen bundles and few elastin fibers; (b) Inner adventitia (IA) is a layered structure with entangled elastin and collagen fibers in each sublayer; (c) The cross section of a coronary artery at no-load state; (e, f) OA and IA deformed under elevated pressures: 100 and 200 mmHg, respectively, showing fibers in IA were stretched to take up loads while most of collagen bundles in OA were still undulated and unengaged; (d) A schematic diagram demonstrates the cross and lateral sections of a vessel segment. (a, c) are the lateral sections and (c, e, f) are the cross sections. Scale bar denotes 100μ.

Figure 2. (a-c) Immunohistochemical images of control and GAGs-digested vessels: (a) Control group (purple); (b) vessel labeled with Antibody CS-56 (brown specifying GAGs); (c) GAGs-digested vessel labeled with Antibody CS-56 (purple confirming GAGs removal); (d) Mechanical testing of coronary adventitia with or without GAGs under physiological axial stretch \( \lambda_z = 1.3 \). There was no significant difference between these two groups (P=0.384).

Figure 3. Comparison of model predictions (solid line) with experimental measurements (dashed line, sample mean, error bar denotes standard deviation of experimental data). The top and lower panels show outer radius and axial force, respectively; at three axial stretch ratios \( \lambda_z = 1.0, 1.3 \) and 1.5.

Figure 4. Model predictions of Cauchy stress components of adventitia (Sample #1) under three axial stretch ratios. Top panel: transmural stress distribution at a fixed luminal pressure 0.013 MPa (100mmHg); Bottom panel: stress components as a function of circumferential strain in the middle wall of the adventitia.

Figure 5. The stress-strain curves of individual elastin and collagen fibers using mean material parameters estimated by various models. Straightening strain \( \varepsilon_0 \) was set to 0.345 for collagen fiber. Capital E and C denote the stress-strain curves of elastin and collagen fibers, respectively. E1 and C1 are the curves estimated by full distribution model with full data sets; E2 and C2 are the curves estimated by full distribution model with partial data sets of \( \lambda_z = 1.3 \) and 1.5; E3 and C3 are the curves estimated by the simplified mean value model with full data sets. \( \sigma_{\theta \theta} \) denotes the circumferential stress-strain relation of the adventitia at middle wall under axial stretch ratio \( \lambda_z = 1.3 \).