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A Microstructurally Motivated Model of Arterial Wall Mechanics with Mechanobiological Implications

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Abstract

Through mechanobiological control of the extracellular matrix, and hence local stiffness, smooth muscle cells of the media and fibroblasts of the adventitia play important roles in arterial homeostasis, including adaptations to altered hemodynamics, injury, and disease. We present a new approach to model arterial wall mechanics that seeks to define better the mechanical environments of the media and adventitia while avoiding the common prescription of a traction-free reference configuration. Specifically, we employ the concept of constituent-specific deposition stretches from the growth and remodeling literature and define a homeostatic state at physiologic pressure and axial stretch that serves as a convenient biologically and clinically relevant reference configuration. Information from histology and multiphoton imaging is then used to prescribe structurally motivated constitutive relations for a bi-layered model of the wall. The utility of this approach is demonstrated by describing in vitro measured biaxial pressure–diameter and axial force–length responses of murine carotid arteries and predicting the associated intact and radially cut traction-free configurations. The latter provides a unique validation while confirming that this constrained mixture approach naturally recovers estimates of residual stresses, which are fundamental to wall mechanics, without the usual need to prescribe an opening angle that is only defined conveniently on cylindrical geometries and cannot be measured in vivo. Among other findings, the model suggests that medial and adventitial stresses can be nearly uniform at physiologic loads, albeit at separate levels, and that the adventitia bears increasingly more load at supra-physiologic pressures while protecting the media from excessive stresses.

Keywords

elastin and collagen; constitutive relation; constrained mixture; residual stress; homeostasis

Introduction

All three primary cell types of the normal arterial wall – endothelial cells of the intima, smooth muscle cells of the media, and fibroblasts of the adventitia – are extremely sensitive to their local mechanical environment^{6,7,32}. Understanding mechanobiological responses by these cells thus requires detailed information on local stress and strain fields, that is, the mechanics of the arterial wall²⁶. Fundamental to such studies is knowledge of the

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Conflicts of interest

None.

constitutive behavior of the wall, which has been the subject of intense research since the early 1970s; see, for example, the reviews by Humphrey²⁵, Vito and Dixon⁴⁵, and Holzapfel and Ogden²³. As revealed by this literature, most prior constitutive relations and stress analyses employ an informal homogenization procedure and treat the wall as a single layer. Such an approach has enabled significant advances, including discovery of important implications of residual stresses⁸ and development of growth and remodeling models that capture salient aspects of arterial adaptations⁴⁴. Nevertheless, stress analyses that account for the different layers of the arterial wall, notably the media and adventitia in all vessels and also the intima in aging and particular diseases, can provide additional information that is essential depending on the question of interest^{2,40}. Indeed, given the recent recognition of differential mechanobiological roles of medial smooth muscle cells and adventitial fibroblasts^{34,17}, there is a pressing need to understand better the layer-specific differences in the local mechanical environments experienced by these different types of cells.

In this paper, we present a new approach for modeling arterial wall mechanics that is motivated by recent growth and remodeling simulations and based on histologically and clinically measurable data. In particular, in contrast to classical approaches that employ either an intact or a radially cut traction-free configuration as a reference, we use the *in vivo* homeostatic state as a biologically and clinically relevant reference configuration and build a new bi-layered model of the arterial wall. Moreover, we endow the primary structurally significant constituents – elastic fibers, smooth muscle, and fibrillar collagen – with individual “deposition stretches,” which ensure that the *in vivo* reference configuration is defined by homeostatic stresses. Embracing the material nonuniformity of the wall distinguishes clearly between a requisite computationally convenient reference configuration for the artery and the actual stress-free (i.e., natural) configurations for the individual constituents. This “constrained mixture” approach allows one to account naturally for tensile stresses in all constituents at physiologic and supra-physiologic pressures as well as for most compressive stresses that necessarily emerge in some constituents at sub-physiologic pressures. The separate prescription of material properties in the media and adventitia is based primarily on histological information on individual mass fractions and orientations of constituents. In addition to achieving fits to *in vitro* biaxial mechanical data that are comparable to prior reports that use classical homogenized models, our new model also allows one to predict associated traction-free configurations, which can serve as independent validations. One advantage of this approach, therefore, is that it does not require one to prescribe residual stress related opening angles, which cannot be measured *in vivo* and cannot be prescribed easily whenever the geometry is not cylindrical. Rather, one merely needs to prescribe point-wise deposition stretches within an assumed homeostatic state regardless of the overall geometry of the wall. The current illustration of this method is based on a cylindrical geometry simply to allow careful comparison with available *in vitro* murine data on biaxial behaviors and traction-free configurations of the common carotid artery.

Methods

Theoretical Framework

Figure 1 illustrates representative cylindrical configurations of an artery that would be available in standard biaxial tests, with the inner layer corresponding to the media and the outer layer to the adventitia. That is, while the intima plays a key role in mechanosignaling, it does not contribute significantly to load bearing in the healthy mouse common carotid artery. The homeostatic configuration κ_h at mean arterial pressure (MAP ~ 93 mmHg) and *in vivo* axial stretch ($\lambda_z^{iv} \sim 1.5$ to 1.6) defines a convenient reference⁵. Cylindrical coordinates denote the position of a material point within the wall in different

configurations: (r, θ, z) for the in vivo, homeostatic reference configuration, (r_p, θ_p, z_p) for any non-homeostatic configuration κ_p at pressure, P and λ_z^{iv} , $(\varrho, \vartheta, \zeta)$ for the intact, traction-free configuration κ_{tf} , and (R, Θ, Z) for the radially cut, traction-free configuration κ_c , wherein the mixture is assumed to be nearly stress-free. Assuming axisymmetry, radial, circumferential, and axial directions are principal directions of strain. The mapping from κ_h to κ_{tf} is given by $\varrho = \varrho(\theta)$, $\vartheta = \theta$, and $\zeta = \frac{z}{\lambda_z}$, whereby

$$[(\mathbf{F}_{tf})_{iI}] = \text{diag} \left[\frac{\partial \varrho}{\partial r} \quad \frac{\varrho}{r} \quad \frac{1}{\lambda_z^{iv}} \right] \quad (1)$$

is the corresponding deformation gradient, with $\lambda_z^{iv} > 1$ the in vivo axial stretch ratio.

Similarly, the mapping from κ_{tf} to κ_c is given by $R = R(\varrho)$, $\Theta = \frac{\pi - \Phi_0}{\pi} \vartheta$, and $Z = \frac{\zeta}{\Lambda_z}$, whereby the deformation gradient is

$$[(\mathbf{F}_c)_{iI}] = \text{diag} \left[\frac{\partial R}{\partial \varrho} \quad \frac{R}{\varrho} \frac{\pi - \Phi_0}{\pi} \quad \frac{1}{\Lambda_z} \right], \quad (2)$$

with $\Lambda_z > 1$ the axial stretch ratio and Φ_0 the opening angle expressed in radians (cf. Fig. 1, noting that such opening angles are meaningful only on a cylindrical geometry). Finally, for the mapping from κ_h to κ_p , $r_p = r_p(r)$, $\theta_p = \theta$, and $z_p = z$, with

$$[(\mathbf{F}_p)_{iI}] = \text{diag} \left[\frac{\partial r_p}{\partial r} \quad \frac{r_p}{r} \quad 1 \right], \quad (3)$$

where the carotid artery is assumed to remain at the in vivo axial stretch when pressurized.

Also following Cardamone et al.⁵, we model the wall as a constrained mixture. Namely, individual structurally significant constituents (e.g., elastic fibers, smooth muscle, and multiple families of collagen fibers) are allowed to possess different natural (i.e., truly stress-free) configurations and material properties, but we assume that they are constrained to move together with the wall. The constituent specific deformations are thus determined, with respect to the individual natural configurations κ_n^α for each constituent α via $\mathbf{F}^\alpha = \mathbf{F} \mathbf{G}_h^\alpha$, where $\mathbf{F}$ represents any deformation of the artery from its homeostatic configuration (e.g., Eqs. 1 to 3) and $\mathbf{G}_h^\alpha$ is a linear transformation associated with the mapping from κ_n^α to κ_h . That is, $\mathbf{G}_h^\alpha$ accounts for cell-mediated deposition stretches when new matrix is incorporated within existent matrix²⁷ as well as, in the case of the elastic fibers, possible stretches induced by development in the absence of subsequent degradation. Incompressibility is assumed for the mixture during transient motions, hence $J = \det \mathbf{F} = 1$, where, as indicated above, we can have $\mathbf{F} = \mathbf{F}_{tf}$, $\mathbf{F} = \mathbf{F}_c \mathbf{F}_{tf}$ or $\mathbf{F} = \mathbf{F}_p$ separately for mappings between κ_h and κ_{tf} , κ_h and κ_p , respectively. Note, too, that $\mathbf{F} \equiv \mathbf{I}$ in the homeostatic, in vivo configuration, where the deformation associated with the in vivo stresses results solely from deposition stretches as it should.

In general, incompressibility allows the radial stretch to be computed in each deformation process via circumferential and axial stretches, while the radial position r occupied by each material point in κ_h is mapped into the other configurations through

$$\varrho = \sqrt{\varrho_i^2 + \lambda_z (r^2 - r_i^2)}, \quad (4)$$

$$R = \sqrt{R_i^2 + \frac{\pi}{\pi - \phi_0} \Lambda_z \lambda_z (r^2 - r_i^2)}, \quad (5)$$

$$r_p = \sqrt{r_{pi}^2 + (r^2 - r_i^2)}, \quad (6)$$

where subscripts i and o denote inner and outer radii, respectively.

For a quasi-static motion in the absence of body forces, balance of linear momentum in its spatial form reduces to $\nabla \cdot \mathbf{t} = \mathbf{0}$, with the Cauchy stress tensor $\mathbf{t}$ obtained as

$$\mathbf{t} = -p\mathbf{i} + 2\mathbf{F} \frac{\partial W}{\partial \mathbf{C}} \mathbf{F}^T, \quad (7)$$

where p is the Lagrange multiplier that enforces incompressibility, $\mathbf{i} = \delta_{ij} \hat{\mathbf{e}}_i \otimes \hat{\mathbf{e}}_j$ is the spatial second order identity tensor, with δ_{ij} the Kronecker delta, W is the strain energy function, $\mathbf{C}$

$= \mathbf{F}^T \mathbf{F}$ is the right Cauchy-Green deformation tensor, and $\bar{\mathbf{t}} = 2\mathbf{F} \frac{\partial W}{\partial \mathbf{C}} \mathbf{F}^T$ is the “extra part” (i.e., deformation dependent) of the stress. The non-zero equilibrium equation, in the radial direction, can be integrated to yield the radial stress in the media (M) or adventitia (A) ²⁴, namely

$$t_{rr}^M(r) = -P_i + \int_{r_i}^r \frac{1}{r} (t_{\theta\theta}^M - t_{rr}^M) dr \quad r_i \leq r \leq r_{MA}, \quad (8)$$

$$t_{rr}^A(r) = -P_i + \int_{r_i}^{r_{MA}} \frac{1}{r} (t_{\theta\theta}^M - t_{rr}^M) dr + \int_{r_{MA}}^r \frac{1}{r} (t_{\theta\theta}^A - t_{rr}^A) dr \quad r_{MA} \leq r \leq r_o, \quad (9)$$

where MA denotes the medial-adventitial interface. Alternatively, integration from r_i to r_o leads to a global radial equilibrium equation in terms of the experimentally measurable transmural pressure, that is, the difference between inner and outer pressures, P_i and P_o . Specifically,

$$\int_{r_i}^{r_{MA}} \frac{1}{r} (t_{\theta\theta}^M - t_{rr}^M) dr + \int_{r_{MA}}^{r_o} \frac{1}{r} (t_{\theta\theta}^A - t_{rr}^A) dr = P_i - P_o. \quad (10)$$

Finally, a global axial equilibrium equation similarly yields the experimentally measurable axial load L

$$\pi \int_{r_i}^{r_{MA}} (2t_{zz}^M - t_{rr}^M - t_{\theta\theta}^M) r dr + \pi \int_{r_{MA}}^{r_o} (2t_{zz}^A - t_{rr}^A - t_{\theta\theta}^A) r dr + \pi (P_i r_i^2 - P_o r_o^2) = L. \quad (11)$$

Note that, like the transmural pressure, the axial load L is non-zero in κ_h and κ_p , but it vanishes in the traction-free configurations κ_{if} and κ_c . An additional condition on the bending moment in the radially cut configuration κ_c requires⁴¹

$$\int_{R_i}^{R_{MA}} R t_{\Theta\Theta}^M dR + \int_{R_{MA}}^{R_o} R t_{\Theta\Theta}^A dR = 0, \quad (12)$$

which, albeit not evident explicitly, allows computation of the experimentally measurable opening angle Φ_0 .

Consistent with the constrained mixture approach²⁷, we assume a mass averaged strain energy function of the form

$$W = \phi^e W^e(\mathbf{F}^e) + \phi^m W^m(\lambda^m) + \sum_{j=1}^4 \phi^{c_j} W^{c_j}(\lambda^{c_j}), \quad (13)$$

where the superscripts e , m , and c_j refer to elastic fiber dominated, smooth muscle dominated, and each of four possible collagen fiber family dominated ($j = 1, 2, 3, 4$) quantities, with ϕ^i and W^i ($i = e, m$, or c_j) the mass fractions and passive strain energy density functions for the constituents that compose the mixture (note: we only consider passive behavior consistent with the available experimental data). $\mathbf{F}^e$ is the deformation gradient tensor experienced by the elastic fibers, and λ^m and λ^{c_j} are stretches experienced by the smooth muscle and the j -th family of collagen fibers (i.e., components of the associated constituent-specific deformation). Rule-of-mixtures relations were first proposed for arteries by Brankov et al.⁴, but we follow Cardamone et al.⁵, which is also consistent with growth and remodeling approaches⁴⁴. Let the behavior of the elastic fiber dominated tissue be described by a neo-Hookean strain energy function^{5,11,22}

$$W^e(\mathbf{F}^e) = \frac{c^e}{2} \left[\text{tr} \left((\mathbf{F}^e)^T \mathbf{F}^e \right) - 3 \right], \quad (14)$$

where c^e is a material parameter with the dimension of a stress and $\mathbf{F}^e = \mathbf{F} \mathbf{G}_h^e$, with $\mathbf{F}$ depending on the specific artery level deformation as noted above. The nonlinear response of collagen fiber (mainly type I) dominated tissue, resulting from the progressive engagement of undulated fibers, is modeled using a Fung-type exponential relationship. Because it is not possible to infer separately the behavior of circumferentially oriented collagen fibers in the media (typically type III) and associated smooth muscle, their combined contributions are similarly modeled using a Fung exponential^{5,35,44} and denoted by m for muscle-dominated. Hence,

$$W^m(\lambda^m) = \frac{c_2^m}{4c_3^m} \left[e^{c_3^m ((\lambda^m)^2 - 1)^2} - 1 \right], \quad (15)$$

and

$$W^{c_j}(\lambda^{c_j}) = \frac{c_2^c}{4c_3^c} \left[e^{c_3^c ((\lambda^{c_j})^2 - 1)^2} - 1 \right]. \quad (16)$$

where c_2^m and c_2^c are material parameters with the dimension of a stress, while c_3^m and c_3^c are dimensionless. Neither the smooth muscle nor the collagen fibers are assumed to have any radial orientation. The stretch experienced by smooth muscle is obtained by projecting $\mathbf{C}$ along the cell axis,

$$\lambda^m = G_h^m \sqrt{\mathbf{C} : (\mathbf{M}^m \otimes \mathbf{M}^m)}, \quad (17)$$

where $\mathbf{M}^m = [0, \sin \alpha_0^m, \cos \alpha_0^m]$ is a unit vector representative of smooth muscle orientation in the reference configuration. Similarly, the stretch in the direction of the collagen fibers is

$$\lambda^{c_j} = G_h^{c_j} \sqrt{\mathbf{C} : (\mathbf{M}^{c_j} \otimes \mathbf{M}^{c_j})}, \quad (18)$$

where $\mathbf{M}^{c_j} = [0, \sin \alpha_0^{c_j}, \cos \alpha_0^{c_j}]$ is a unit vector that identifies the dominant orientation of the j -th family of collagen fibers.

In addition to the percentage of the wall thickness occupied by the media (γ_{MA}) as well as the external radius (r_o) and the internal and external pressures (P_i, P_o) in κ_h , other parameters required as input to the model are the mass fractions (ϕ^e, ϕ^m, ϕ^f), the orientation of smooth muscle and collagen fibers ($\alpha_0^m, \alpha_0^{c_j}$), the deposition and developmental stretches ($\mathbf{G}_h^e, G_h^m, G_h^{c_j}$), the parameters describing the passive mechanical behavior of individual constituents ($c^e, c_2^m, c_3^m, c_2^c, c_3^c$), and the internal and external pressures in any configuration κ_p . Based on the data provided as input, the Newton-Raphson method was used to determine the unknown parameters: internal radius (r_i, r_{pi}) and total axial force (L_h, L_p) in κ_h as well as any κ_p , internal radius (ϱ_i) and axial stretch (λ_z) in κ_{if} , and internal radius (R_i), axial stretch (A_z) and opening angle (Φ_0) in κ_c . At each iteration, the radius in κ_h corresponding to the medial-adventitial interface is determined as $r_{MA} = r_i + \gamma_{MA}(r_o - r_i)/100$. Also, Eqs. (4), (5) and (6) are used to compute $\varrho_o, \varrho_{MA}, R_o, R_{MA}, r_{po}$ and r_{pMA} .

Experimental methods

A 10-week old C57BL/6 wild-type mouse was euthanized with an overdose of sodium pentobarbital (250 mg/kg), consistent with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee. The left common carotid artery was excised, cleaned of the excess perivascular tissue, and equilibrated in Hanks balanced salt solution (HBSS), which renders the vessel passive. Rings approximately 0.5 mm long were sliced from the vessel and opened by means of a single radial cut. Images of the $N = 10$ rings were taken with a dissection microscope at 40x magnification before and after the cut, once a steady configuration was reached (~5 minutes). The images were analyzed with a custom, semi-automatic method implemented in MATLAB (Mathworks, Inc., Natick, MA) to measure the opening angle Φ_0 . Three ($n = 3$) additional 10-week old C57BL/6 wild-type mice were euthanized similarly and the left common carotid artery was excised, cleaned, and cannulated on glass pipettes for mechanical testing using a custom biaxial device¹⁸ according to a previously published protocol¹⁴. After four cycles of preconditioning, cyclic pressure-diameter tests were performed in HBSS at three axial extensions (λ_z^{iv} and $\lambda_z^{iv} \pm 5\%$) and cyclic axial force-length tests were performed at three pressure levels (60, 100 and 140 mmHg). Internal pressure, external diameter in the central region, axial force, and axial extension were measured online.

Parameter Estimation

Consistent with a structurally motivated approach, many parameters were prescribed based on histological measurements, while others were estimated via empirical inferences or nonlinear regression of biaxial data. Toward this end, intact arterial segments from specimens excised for opening angle experiments or inflation-extension experiments were prepared for histological analyses following a previously reported protocol^{12,15}. Sections were stained with Hematoxylin & Eosin (H&E), Verhoeff-Van Gieson (VVG), or Masson's Tri-Chrome (MTC) to assess the morphology of cells and extracellular matrix structures as well as to quantify the fraction of total area occupied by specific constituents^{12,43}. Slides were observed at 20x magnification using an Olympus BX/51 microscope (Olympus Inc., Center Valley, PA) and imaged using an Olympus DP70 digital camera. Custom MATLAB routines^{3,12} were used to separate elastic fibers, smooth muscle, and collagen fibers based on hue, saturation, and lightness (HSL) thresholding. The area fraction for elastin was computed as the ratio between the pixels classified as elastic material and the total pixels

recognized as tissue in VVG-stained slides. A similar procedure was followed to calculate the area fraction for smooth muscle and collagen fibers from MTC-stained slides. Assuming a constant mass density and that the relative abundance of microstructural constituents does not vary axially, the area fractions for elastic fibers, smooth muscle, and collagen fibers were considered as estimates of the corresponding mass fractions ϕ^e , ϕ^m and ϕ^c . Finally, manual thresholding was used to crop the adventitia from VVG and MTC images. The total count of tissue pixels from the image after thresholding relative to that in the original image served to estimate the percentage of the cross-sectional area occupied by the media in the intact, traction-free configuration.

Unlike many other parameters of the model, deposition stretches cannot be measured or estimated easily from data. Hence, experimental measures of circumferential and axial stretches under physiologic loads combined with data from the literature reporting the effect of selective enzymatic degradation of the extracellular matrix on vessel geometry were used to identify reasonable ranges for G_h^e , G_h^m , and $G_h^{c_j}$. Fine-tuning of these parameters was then performed to best fit the experimental data. For example, previous studies reveal that functional elastin is synthesized primarily during the perinatal period and it remains stable for long periods afterwards⁹. Elastic fibers within the arterial wall are thus expected to stretch due to somatic growth. Independent experimental investigations show that the elastic network remains under tension upon removal of external loads and even in the open configuration after a radial cut^{37,53} (which is thus not truly stress-free as it is often assumed). Consistent with these observations, deposition stretches for the elastic fibers in the circumferential and axial directions were assigned values approximately 5-10% above the highest circumferential and axial stretches experienced by the media during inflation-extension tests between κ_{ff} and κ_h . Specifically, in the case of the three tested vessels, deposition stretches ranged from $G_{h\theta}^e \in [2.02, 2.77]$ and $G_{hz}^e \in [1.61, 1.63]$, with

$G_{hr}^e = \frac{1}{G_{h\theta}^e G_{hz}^e} \in [0.22, 0.31]$ for incompressibility. Conversely, collagen has a half-life of ~70 days and it is continuously deposited and degraded throughout life³⁰. Although collagen fibers support tensile loads in vivo, considerable data suggests the ability of proteoglycan-supported circumferential collagen¹⁰ to support compressive loads in both the intact and cut traction-free configurations^{16,37,38,53}. Recently, Ferruzzi et al.¹⁵ quantified the effect of elastase treatment on unloaded outer diameter and unloaded axial length of common carotid arteries from wild-type mice. Axial and circumferential stretches between the treated and untreated configurations were taken as a first estimate for the stretches between the natural stress-free configuration of collagen fibers and smooth muscle and the intact traction-free configuration of the vessel κ_{ff} . These values were then multiplied by the lowest circumferential and axial stretches experienced by media and adventitia during inflation-extension tests between κ_{ff} and κ_h to estimate ranges for the deposition stretches. To improve the fitting of the experimental data, the circumferential and axial stretches inferred from Ferruzzi et al.¹⁵ were increased by 15-20%, leading to deposition stretches for the three vessels within the range $G_h^{c_1} \in [1.11, 1.13]$ for axial collagen fibers, $G_h^{c_{3,4}} \in [1.11, 1.12]$ for diagonal collagen fibers, and $G_h^{c_2} = G_h^m \in [1.07, 1.09]$ for both circumferential collagen fibers in the adventitia and smooth muscle with associated circumferential collagen fibers in the media.

Mass fractions (ϕ) estimated from histological assays were prescribed as known parameters. Smooth muscle was assumed to have only a circumferential orientation ($\alpha_0^m = \pi/2$). Four families of mechanically-equivalent collagen fibers were included, the first parallel to the axis of the artery ($\alpha_0^{c_1} = 0$), the second oriented in the circumferential direction ($\alpha_0^{c_2} = \pi/2$), and the third and fourth oriented diagonally and symmetrically with respect to the vessel

axis ($\alpha_0^{c4} = -\alpha_0^{c3} = \alpha_0^c$). The fractions of total collagen oriented axially and circumferentially were indicated respectively with β^x and β^θ , while the fraction of total collagen oriented diagonally β^l satisfied $\beta^x + \beta^\theta + 2\beta^l = 1$. The contribution of each of the four families of collagen fibers to the strain energy function in Eq. (13) was thus weighted by the product of the total collagen mass fraction (ϕ^c) with β^x , β^θ or β^l , namely $\phi^1 = \beta^x \phi^c$, $\phi^2 = \beta^\theta \phi^c$ and $\phi^{3,4} = \beta^l \phi^c$. Note that 97% of total elastin, 100% of total smooth muscle, and 15% of total axial and diagonal collagen fibers were assigned to the media. Conversely, 3% of total elastin, 85% of total axial and diagonal collagen, and 100% of total circumferential collagen were assigned to the adventitia. The potential contribution of circumferential collagen fibers in the media was combined with smooth muscle as neither histological nor mechanical data were sufficiently informative to separate the contributions of these two constituents; moreover, much of the medial collagen tends to be type III, not type I as found in the adventitia, hence this weighting helped reduce the effective stiffness of the medial collagen.

Best-fit values of the yet unknown model parameters were then determined from the biaxial data via nonlinear, least-squares regression using the objective function proposed by Wan et al.⁴⁹, namely

$$e = \sum_{i=1}^M \left[\left(\frac{P^{th} - P^{exp}}{\bar{P}^{exp}} \right)_i^2 + \left(\frac{L^{th} - L^{exp}}{\bar{L}^{exp}} \right)_i^2 \right], \quad (19)$$

where M is the total number of data points, the superscripts *exp* and *th* indicate experimentally measured and theoretically predicted values of pressure P and axial force L , respectively, and the overbars denote mean values. Toward this end, based on values of circumferential stretch registered during the inflation-extension tests and on the deposition stretches assigned to the smooth muscle and collagen fibers, the experimental data were split *a priori* into two sets. The first set included the configurations of pressurization and axial extension where all the constituents of the arterial wall experienced tension; the second set collected all remaining data points wherein either proteoglycan-supported collagen fibers or smooth muscle experienced compression. This split occurred between 40 and 80 mmHg for the $n = 3$ different data sets. Eight parameters were estimated from the first data set: c^e , $c_2^{m_t}$, $c_3^{m_t}$, $c_2^{c_t}$, $c_3^{c_t}$, α_0^c , β^θ and β^x , where subscript t indicates tensile behavior. These parameters were constrained to be non-negative and $\alpha_0^c \leq \pi/2$ for the symmetry of the diagonal fibers. These estimated parameters describing the tensile responses were then fixed in the additional regression performed on the second dataset, where the material properties of collagen fibers and smooth muscle in compression were assessed. Assuming a similar response of the two elements in compression (due to a lack of information otherwise), only two parameters were estimated: $c_2^{c_c} = c_2^{m_c}$ and $c_3^{c_c} = c_3^{m_c}$, with the subscript c referring to compressive behavior, both required to be non-negative.

Results

Experimental and Constitutive

The mean ($\pm$ standard deviation, $N = 10$ rings) opening angle was $\Phi_0 = 29 \pm 8$ deg. The mean ($\pm$ standard deviation, $n = 3$ carotid arteries) values of geometrical parameters and in vivo axial stretch from inflation-extension tests are in Table 1, while mean ($\pm$ standard deviation, $N = 10$ rings) geometrical measures from opening angle experiments are in Table 2.

Figure 2 shows representative cross sections stained with VVG and MTC, together with the masks that separated the primary load-bearing constituents based on HSL values of each

pixel. Mean estimated area mass fractions were $\phi^e = 0.249 \pm 0.018$ for elastic fibers, $\phi^m = 0.279 \pm 0.033$ for smooth muscle associated with circumferential collagen fibers in the media, and $\phi^f = 0.458 \pm 0.025$ for all collagen fibers ($N = 10$ histological images). The average percentage of the wall occupied by the media in the intact, traction-free configuration was $\gamma_{MA} = 45 \pm 4\%$ ($N = 10$ histological images). Figure 3 compares best-fit theoretical results (solid symbols / lines) with experimental data (open symbols) from cyclic pressure-diameter tests at three axial stretches for a representative specimen (S1): pressure vs. outer diameter and axial force vs. pressure. Notice the slight change in slope in the pressure – diameter prediction, that is, the “transition” between configurations wherein all components were extended (fit based on dataset 1) and those wherein smooth muscle and collagen fibers were at least in part compressed (fit based on dataset 2); this transition was likely due to the finite number of fiber families in the model. Based on work by Wilson et al.⁵², the theoretically modeled transition would be expected to be smoother when implementing the same model within a growth and remodeling framework for tissue maintenance, since myriad cohorts of each fiber family arise in tissue maintenance due to the continuous turnover of constituents. Regardless, Table 3 lists the three sets of structural and mechanical parameters estimated by nonlinear regressions of the biaxial data for the $n = 3$ carotids. Projecting iso-energy contours for W onto the $\lambda_\theta - \lambda_z$ plane confirmed the convexity of this potential for both the media and the adventitia over the full range of deformations considered, including compressive, and further revealed differences in energy storage, stiffness, and anisotropy for these two layers (Fig. 4). Note that the circumferential stretch measured between κ_h and κ_0 was assigned as a lower limit in this figure while the circumferential stretch between κ_h and κ_{220} (i.e., at 220 mmHg pressure) was used as an upper limit.

Predicted Results

Table 1 also reports mean ($\pm$ standard deviation, $n = 3$) absolute percent errors for the model predictions relative to experimental measurements of geometrical parameters (inner radius and thickness), axial stretch, and axial force in the homeostatic κ_h and intact, traction-free κ_{tf} configurations for the arteries tested with the biaxial device. The mean percentage errors remained below 7%, hence providing an additional level of validation for the modeling approach. Table 2 also compares the mean ($\pm$ standard deviation, $N = 10$) inner radius, thickness, and opening angle predicted by the model for the cut, traction-free configuration κ_c with the experimental results from opening angle tests. Although the opening angle experiments ($N = 10$) were performed on rings from a vessel different from those ($n = 3$) tested biaxially, the agreement was good nonetheless.

Stress Analysis

Figure 5 shows the transmural distribution of Cauchy stress predicted for the mixture κ_h for one set of estimated parameters. The radial stress was continuous across the wall and primarily reflected the traction boundary conditions, increasing monotonically from negative internal pressure at the inner radius to zero at the outer radius. In contrast, the circumferential and axial stresses were discontinuous at the interface between the media and adventitia. Although identical constituents, endowed with the same mechanical response, populated the two layers of the wall, their relative abundance and orientations differed, thus endowing the media and adventitia with very different overall material properties (cf. Fig. 4). The circumferential and axial stresses in the homeostatic configuration κ_h were almost uniform in both layers, though their values differed for the media and adventitia. This result suggested that the smooth muscle of the media and the fibroblasts of the adventitia may have different mechanobiological targets and homeostatic tendencies.

Figure 6 shows transmural distributions of the extra stress $\bar{\mathbf{t}}$ for the individual constituents of the mixture in κ_{ff} , κ_h , and one additional configuration κ_{180} (i.e., at 180 mmHg) based on the same representative set of estimated parameters. According to model predictions, based on the same representative set of estimated parameters. According to model predictions, the elastic network remained extended (i.e., in tension) in κ_{ff} after removal of the external loads, while the collagen fibers and smooth muscle were compressed. In contrast, all constituents were in tension in κ_h , wherein the circumferential stress generated by the internal pressure was supported mainly by the elastic fibers in the media, with the smooth muscle and circumferential collagen fibers less engaged. A more consistent tensile contribution was provided by the circumferential and diagonal families of collagen fibers in the adventitia. The axial stress associated with the axial extension of the artery was almost equally shared between the elastic fibers of the media and the axial and diagonal collagen fibers in the adventitia. A pressure rise at constant in vivo axial stretch only marginally affected the stress state of the elastic fibers, suggesting that other constituents carried the additional mechanical load. Indeed, an increase in pressure above normal caused the circumferential and diagonal collagen fibers in the adventitia to engage, resulting in a dramatic increase in the stress sustained by these constituents, while “protecting” the constituents of the media. That is, there was only a modest rise in the contribution of the smooth muscle and associated circumferential collagen fibers in the media.

The partitioning of loads amongst constituents, upon changes in transmural pressure at a constant in vivo axial stretch, also affected the transmural distribution of stress at the gross level. Figure 7 shows the circumferential component of Cauchy stress predicted by the model at four different pressure levels, from 100 mmHg to 220 mmHg, for the same representative set of estimated parameters. While the circumferential stress was supported primarily by the media within the normal physiologic range of pressures, the adventitia carried increasingly more load as the pressure progressively rose above normal. Indeed, the circumferential stress was higher in the adventitia than in the media for pressures above 180 mmHg. This result agrees with previous speculations that elastic laminae in the media provide compliance and resilience at physiologic pressures and the collagen fibers in the adventitia act to protect the wall against acute over-distensions at higher pressures.

Discussion

As noted earlier, single-layered homogenized models of arterial wall mechanics have provided, and will continue to provide, important insight into arterial function, including mechanical homeostasis. Karsaj et al.²⁹ showed, for example, that single- and bi-layered growth and remodeling models can provide the same information on the evolving geometry and structural stiffness, which is sufficient for many fluid-solid-growth (FSG) models²⁸. Indeed, because residual stresses tend to homogenize transmural distributions of wall stress, single- and bi-layered models can provide comparable estimates of overall wall stress, the mean value of which is dictated by the universal solution known as Laplace’s relation (cf. Fig. 5). Because of the different mechano-responsiveness of medial smooth muscle cells and adventitial fibroblasts^{6,32}, however, there is a need to distinguish possible differences in homeostatic stresses in the media and adventitia.

The first bi-layered models of the arterial wall based on the theory of finite elasticity were proposed by von Maltzahn and coworkers^{46,47}. They reported discontinuous circumferential and axial stresses at the interface between the media and adventitia, with maximum circumferential values highest in the media in the later paper. These stresses were far from uniform within the two layers, however, mainly because residual stresses were neglected. Among the more recent studies, Holzapfel et al.²² also used a bi-layered model to study transmural distributions of stress (cf. Figure 18 in their paper). They showed that

circumferential stress was much higher than axial stress in the media, but axial stress was slightly higher in the adventitia at 100 mmHg. Despite including a residual stress related opening angle, neither the circumferential nor the axial stress was uniform within the media. Holzapfel and Gasser²⁰ further reported a finite element model for studying balloon angioplasty based on an approach similar to their 2000 paper. Different values of material parameters for the media and adventitia yielded much lower and nearly uniform, layer-specific circumferential stresses under physiologic conditions of pressure and axial stretch. In contrast, Rachev³⁶ and Taber and Humphrey⁴¹ used bi-layered models within growth and remodeling simulations. The former was based on the hypothesis that mean circumferential stress is restored to normal whereas the latter was based on the hypothesis that local circumferential stress was restored to normal. Each study reported a uniform, or nearly so, distribution of circumferential stress in both the media and adventitia following adaptation. Of particular note, Taber and Humphrey⁴¹ suggested that transmural differences in material properties were needed to recover experimentally observed opening angles, which in turn contributed to the more uniform transmural distributions of stress.

Similar to results in Holzapfel et al.²², we found higher circumferential stresses in the media than in the adventitia at physiologic pressures. In contrast, however, the present model suggested further that circumferential and axial stresses can be uniform at different values in the two layers, which appears favorable mechanobiologically. That is, smooth muscle cells could experience the same net mechanical environment regardless of their radial position within the media and similarly for fibroblasts within the adventitia, though each cell type could have a different mechanobiological target. In addition, the present model suggested that the primary load carrying capability could switch dramatically from the media at physiologic pressures to the adventitia at supra-physiologic pressures – recall Fig. 7 wherein medial stresses increased ~2x while adventitial stress increased ~7x due to the prescribed 2.2-fold increase in pressure. These results are consistent with the concept that the adventitia may serve as a protective sheath that prevents overall acute over-distension while stress shielding the underlying parenchymal layer. Indeed, Eberth et al.¹³ found that the primary growth and remodeling response of the mouse common carotid artery in banding-induced hypertension was a marked increase in adventitial collagen, which would be consistent with a marked increase in adventitial stress locally upregulating pro-fibrotic cytokines or growth factors.

Figure 6 suggested further that the deformation-dependent (i.e., extra) part of the stress increased proportionally less in the elastic network within the media than it did in the collagen fibers in the adventitia in response to a near doubling of pressure. Results for the medial smooth muscle + circumferential collagen could not be interpreted easily because of the lack of information on the partitioning of load between these two constituents. Although there was a predicted significant fold increase in stress in the medial smooth muscle + collagen, the absolute value remained less than a modest 40 kPa. The model also predicted a tensile, deformation-dependent part of the stress acting on the elastic network in the radial direction (~5 to 10 kPa). The existence of a constituent-specific tensile radial stress, despite an overall compressive radial stress (cf. Fig. 5), could be consistent with an intralamellar swelling due to the presence of chondroitin sulfate proteoglycans that fill the intralamellar space and maintain separation between adjacent laminae¹⁰. There is a need to explore this possibility further. For example, tensile radial stresses in the elastic network could enable smooth muscle cells to sense changes in tensile loading through observed radial connections with the network of elastic fibers. Clearly, however, much work remains to model the complex mechanics and mechanobiology of smooth muscle cells within a 3-D matrix. For example, additional experiments or different regression strategies should be designed to estimate separately the passive response of smooth muscle and the behavior of circumferential collagen fibers in the media. Moreover, the multiaxial active contraction of

smooth muscle cells should be included to account for its contribution in vivo^{1,48}; this was not explored herein because of the use of in vitro data for passive arteries.

The concept of residual stress in arteries has been universally accepted since publication of the seminal paper by Chuong and Fung⁸, yet few have discussed implications of the requisite self-equilibrating compressive and tensile circumferential stresses that must exist in the excised intact configuration. That is, most studies simply compute these compressive stresses using constitutive relations that were derived from pressure – diameter tests (i.e., tacitly assumed tensile behavior). Moreover, most simply assume that collagen fibers within so-called “fiber family” models cannot support compression²², an assumption that dates back to the seminal paper by Lanir³¹. Histology reveals, however, that collagen fibers do not exist as thin isolated structures within the wall. Rather, they interact with each other and other structurally significant constituents, including glycosaminoglycans (GAGs) and proteoglycans (PGs). In particular, aggregating GAGs / PGs appear to insert within undulated collagen fibers perpendicular to the local fiber direction¹⁰, which would allow the collagen fiber – GAG / PG complex to support compressive loads (e.g., not unlike a laterally supported Euler column). Indeed, this concept simply expands the view that aggregates of hyaluronan and versican within the media endow the wall with a significant ability to resist radial compressive loads⁵⁰. Although structurally motivated, we must remember that current two- and four-family constitutive models are phenomenological nonetheless (e.g., parameter values are determined via regressions using whole artery data), hence the yet to be modeled but inherent contributions of GAGs / PGs must be captured via the best-fit parameters. There is clearly a need for much more attention to this important issue. Martufi and Gasser³³ proposed a multiscale constitutive relation for passive arteries to model contributions of PGs to the cross-linking of collagen fibrils, but they did not consider compressive behaviors.

We did not explicitly introduce contributions of GAGs / PGs into the overall mechanical response of the arterial wall. Rather, the behavior of proteoglycan-supported collagen fibers was allowed by using a bi-modular constitutive relationship, with different material parameters in tension and compression⁵. The existence of a compressive behavior is consistent with the aforementioned presence of residual stresses and, in particular, the associated separate roles of elastin and collagen^{5,19,53}. Indeed, we suggest that sub-physiologically loaded arteries, not just excised traction-free segments, likely manifest self-equilibrating compressive and tensile loads (cf. Fig. 1). Our estimate of a progressive transition from compressive to tensile behavior within the pressure range 40-80 mmHg is consistent with recent findings from confocal imaging on a pressure related transition of collagen fibers from undulated to straight³⁹. Although we suggest that it is imperative for our field to address better this issue of compressive behavior, one of the implicit advantages of our choice of a homeostatic reference configuration is that we actually avoid this conundrum that is otherwise inherent to all opening angle based stress analyses. That is, we can easily focus on in vivo mechanics at physiologic and supra-physiologic pressures where all constituents are always in tension in circumferential and axial directions.

Nevertheless, considering the compressive behavior allowed us to predict traction-free configurations that can be measured in vitro, and thereby to seek an additional level of validation of the overall model. Admitting a compressive behavior of the collagen fiber – GAG / PG complex introduces implications on a theoretical level. Plotting iso-energy contours (cf. Fig. 4) allows one to assess the local convexity of a strain energy function²², but convex contours represent only a necessary, not sufficient, condition⁵¹. Figure 4 was nevertheless consistent with convexity even in compression. Holzapfel et al.²¹ discussed conditions for strong ellipticity of fiber-family models. Although they noted that “it is not easy to find necessary and sufficient conditions,” they provided a method to test ellipticity. With regard to fiber-family models, they concluded that “strong ellipticity is therefore

consistent with fibre extension ... [but] ... strong ellipticity could still hold if there is some compression in the fiber direction.” Using their method, we found that the current model remained strongly elliptic, for the parameters values listed, for all intact configurations (cf. Fig. 1). Although not studied in detail, retaining strong ellipticity in configurations experiencing compression may have been aided by the presence of circumferential and axial fiber families in addition to the diagonal families considered by Holzapfel and colleagues. Regardless, our findings on convexity and ellipticity suggest that the goodness of the predictions in Table 1 represent a strong model validation; the reasonable predictions in Table 2 for the radially cut configurations, despite a discovered possible loss of ellipticity in that state, are also encouraging.

We conclude, therefore, that the present model offers numerous advantages. One can use a biologically and clinically relevant in vivo reference configuration that captures beneficial effects of residual stresses while avoiding the need to measure or prescribe opening angles, which cannot be obtained in vivo and cannot be defined easily on non-cylindrical geometries. One can focus on in vivo behaviors wherein structurally significant constituents are always in tension in the circumferential and axial directions and thereby avoid the continuing lack of data on compressive behavior. And, one can begin to address the partitioning of load not just by layer, but also by constituent. That said, there is clearly a need to continue our search for improved constitutive relations that, first, account directly for experimental observations that proteoglycan-supported collagen fibers and smooth muscle likely support compressive loads^{16,37,38,53}, second, guarantee convexity in both tension and compression via appropriate constraints on the values of the parameters, and third, ensure strong ellipticity to enable future finite element implementations. Indeed, such a search should seek to overcome the mathematically convenient, but unphysical, assumption of an uncoupled matrix and fibers (i.e., the additive split of the stored energy). Overall, this search must also be guided by a desire to understand both the mechanobiology (e.g., ability of the wall to grow and remodel) and the structural functionality, not just the mere best-fitting of data. The present model suggested that the medial smooth muscle cells and adventitial fibroblasts may have different mechanobiological target stresses, with the adventitia carrying increased loads at supra-physiologic pressure that could stimulate the fibroblasts to contribute significantly to the associated adaptive responses. Such a possibility is supported by diverse experimental observations^{17,42}. Indeed, extending the present model to growth and remodeling simulations of adaptations, responses to injury, and disease progression^{5,44} should remain our ultimate goal.

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FIGURE 1.

Schematic illustration of some of the key configurations that were used in the nonlinear regression or used to test the predictive ability of the constitutive formulation for the mouse carotid artery. κ_h : homeostatic configuration at a mean arterial pressure of 93 mmHg and in vivo axial stretch $\lambda_z^{iv} = 1.5$ to 1.6. κ_p : loaded configurations at λ_z^{iv} and any pressure P , with representative values shown for $P = 0, 60, 180$ mmHg. κ_{tf} : intact, traction-free configuration. κ_c : radially cut, traction-free configuration. κ_n : natural (i.e., stress-free) configurations for individual constituents. All configurations are shown on a graph where the transmural pressure is plotted against the current outer diameter (od) as predicted by the numerical model, relative to the outer diameter in the intact, traction-free configuration κ_{tf} . Note, for example, that the κ_0 configuration is unpressurized but axially stretched, hence its diameter is less than that in the intact traction-free configuration.

FIGURE 2.

Illustrative cross-sections from a wild-type mouse carotid artery stained with Verhoeff-Van Gieson (VVG, top, left) and Masson's Tri-Chrome (MTC, top, right) and imaged at 20x magnification. The bottom masks separate the primary load-bearing constituents based on the hue, saturation and lightness (HSL) values pertaining to each pixel. In addition to the usual observations (e.g., that elastin and smooth muscle are confined primarily to the media and the adventitia consists mainly of dense collagen), note the thin layers of collagen that adjoin the elastic laminae in the media.

FIGURE 3.

Representative best-fit (solid symbols / lines) to pressure vs. outer diameter and axial force vs. pressure data (open symbols) collected during cyclic pressure-diameter testing at three different axial stretches, λ_z^{iv} and $\lambda_z^{iv} \pm 5\%$, for one of the three arteries tested. This fit was obtained using the constitutive relationship in Eq. (13) and objective function in Eq. (19).

The symbol * shows the selected in-vivo, homeostatic state ($P \sim 93$ mmHg and $\lambda_z^{iv} \sim 1.55$). Note that the predictions in the pressure – diameter plot reveal a slight change in slope (between 60 and 80 mmHg), which signals the assumed transition from possible compression and tension in the collagen fibers to tension only in these fibers (cf. Table 3).

FIGURE 4.

Representative iso-energy contour plots of W in the plane $\lambda_\theta - \lambda_z$ for the media (*upper panel*) and the adventitia (*lower panel*), with the ranges of stretch chosen to cover all deformations that were included in the stress analyses. In contrast with most formulations, note that the reference configuration is the in vivo homeostatic configuration, with components of $\mathbf{F}$ given by $(\lambda_\theta, \lambda_z) = (1, 1)$ as indicated by the *. This plot confirms that the media bears most of the load in the homeostatic configuration and shows the different anisotropies in the media and adventitia based on our best-fit values of the material parameters. Finally, these contours confirm convexity of W even in compression; the superimposed numerical values on the contours denote the values of strain energy in kPa.

FIGURE 5.

Predicted transmural distributions of radial (dash-dotted), circumferential (solid), and axial (dashed) components of Cauchy stress in the homeostatic configuration κ_h , at mean arterial pressure (MAP ~ 93 mmHg) and $\lambda_z^{iv}=1.5$, based on one representative set of estimated parameters. The mean circumferential stress, as obtained from Laplace's relation, is shown for comparison (dotted horizontal line). All components of stress are plotted as a function of the normalized current radius, with 0 and 1 corresponding to inner and outer radii, respectively. The vertical light grey solid line identifies the border between the media and adventitia, at approximately 0.42.

FIGURE 6.

Representative transmural distributions of components of the extra stress $\bar{\mathbf{t}}$ for the elastic network, collagen fibers, and smooth muscle plus associated circumferential (c) collagen fibers, all for three different loading configurations. *Upper panel:* intact, traction-free configuration κ_{tf} . *Middle panel:* homeostatic configuration κ_h at mean arterial pressure and λ_z^{iv} . *Lower panel:* elevated pressure configuration κ_{180} at 180 mmHg and λ_z^{iv} . Similar to Figure 5, stress is plotted as a function of the normalized radius $\in [0,1]$. The vertical light grey solid line that locates the interface between the media and adventitia falls approximately at 0.45 in κ_{tf} and at 0.42 in both κ_h and κ_{180} .

FIGURE 7.

Representative transmural distribution of Cauchy stress in the circumferential direction for four different pressures above homeostatic: 100 mmHg (dash-dotted line), 140 mmHg (dotted line), 180 mmHg (solid line), and 220 mmHg (dashed line). Similar to Fig. 5, stress is plotted as a function of the normalized radius $\in [0,1]$. The media/adventitia boundary occurs approximately at 0.42. Note the much more dramatic increase in adventitial stress.

TABLE 1

In-vivo and intact, traction-free configurations.

		Experimental Measurements	Predictions (% Errors)
r_i	(μm)	299 ± 26	0.7 ± 0.6
h_h	(μm)	26 ± 5	6.3 ± 1.2
f_h	(mN)	4.0 ± 1.2	5.3 ± 3.1
ϱ_i	(μm)	128 ± 33	3.0 ± 1.0
h_{tf}	(μm)	76 ± 18	5.3 ± 4.7
λ_z^{iv}	(-)	1.55 ± 0.01	0.7 ± 0.6

Mean ($\pm$ SD, $n = 3$) experimental measurements during inflation-extension tests and mean ($\pm$ SD, $n = 3$) absolute percentage errors of model predictions with respect to the experimental data: r_i , the inner radius in κ_h , ϱ_i , the inner radius in κ_{tf} , λ_z^{iv} , the in vivo axial stretch, h , wall thickness, and f , the axial force, with subscripts h and tf referring to the configurations κ_h and κ_{tf} , respectively.

TABLE 2

Cut, traction-free configuration.

		Measurements	Predictions
R_i	(μm)	148 ± 23	147 ± 35
H	(μm)	60 ± 4	77 ± 19
Λ_z	(-)	-	1.0 ± 0.0
Φ_0	(deg)	29 ± 8	21 ± 3

Mean experimental measurements ($\pm$ SD, $N = 10$ rings from a single artery) for opening angle tests compared with mean model predictions ($\pm$ SD, based on results for $n = 3$ different arteries) in the cut, traction-free configuration κ_C : R_i , the inner radius, H , wall thickness, Λ_z , the axial stretch between κ_C and κ_{tf} , and Φ_0 , the opening angle.

TABLE 3

Mechanical properties.

		S1	S2	S3
Tension				
c^e	(kPa)	337.97	63.72	72.32
$c_2^{m,t}$	(kPa)	32.00	11.30	7.85
$c_3^{m,t}$	(-)	4.20	4.55	2.62
$c_2^{c,t}$	(kPa)	1383.98	1251.45	1445.08
$c_3^{c,t}$	(-)	6.42	8.17	9.97
α_0^c	(deg)	36.37	40.27	42.63
β^θ	(-)	0.05	0.03	0.04
β^z	(-)	0.12	0.06	0.09
RSME	(-)	0.05	0.07	0.07
Compression				
$c_2^{m,c} = c_2^{c,c}$	(kPa)	18.61	16.16	14.86
$c_3^{m,c} = c_3^{c,c}$	(-)	1.77	5.32	4.65
RSME	(-)	0.09	0.09	0.10

Structural and mechanical parameters estimated for the $n = 3$ specimens, S1 to S3, that were tested with the biaxial device: c^e , the constitutive parameter for the elastin-dominated tissue, $c_2^{m,t}$ and $c_3^{m,t}$, the constitutive parameters for the smooth muscle and associated circumferential collagen fibers in tension, $c_2^{c,t}$ and $c_3^{c,t}$, the constitutive parameters for the collagen fibers in tension, $c_2^{m,c} = c_2^{c,c}$ and $c_3^{m,c} = c_3^{c,c}$, the constitutive parameters for the smooth muscle and the families of collagen fibers in compression, α_0^c the reference orientation of diagonal collagen with respect to the axial direction, β^θ and β^z , the fraction of total collagen oriented in the circumferential and axial directions. Goodness of fit is provided in terms of root mean square error RMSE.