Micromechanically-motivated analysis of fibrous tissue

M. Ben-Or Franka, J.A. Niestrawskab, G.A. Holzapfelb,c, G. deBottona,d,∗

a

Department of Biomedical Engineering, Ben-Gurion University, Beer-Sheva, 84105, Israel

b

Institute of Biomechanics, Graz University of Technology, 8010, Graz, Austria
c

Department of Structural Engineering, Norwegian University of Science and Technology (NTNU), 7491, Trondheim, Norway
d

Department of Mechanical Engineering, Ben-Gurion University, Beer-Sheva, 84105, Israel

ARTICLE INFO

Keywords:

Fibrous tissue

Media

Collagen fiber

Anisotropy

Periodic homogenization

ABSTRACT

Collagen fibers are the main load bearing component in fibrous tissues. Systematic analyses of their structure and orientation are thus crucial for the development of material models that enable to predict the mechanical tissue response. To this end, biaxial tests at different stretch ratios were performed on two tissue samples of the medial layer extracted from a human aorta. The tissues were loaded in the circumferential and axial directions simultaneously. We develop here a micromechanical model which is based on structural parameters of collagen fibers that were extracted from second-harmonic generation images of the two samples. The tissue is modeled as a periodic six-layered laminate in which the individual layers are treated as periodic fibrous structures with one family of fibers. We make use of the Hill-Mandel theory in the context of periodic homogenization to determine the overall mechanical tissue response. Both the analytical and numerical models are able to capture the overall mechanical response of the two tissue samples using a straightforward representation of the tissue structure together with a limited set of material parameters. Up to 10% of strains the model captures the almost linear response of both tissue samples. Beyond that stretch level the stiffening of the tissues becomes more evident, especially in the circumferential direction. In cases where the axial stretch is larger than the circumferential stretch the predictions are somewhat stiffer, while a very good agreement is obtained when the circumferential stretch is dominant. The stiffening of one tissue sample was substantially larger than the other, implying that higher-order stiffening mechanisms may kick in at larger strains. Our sensitivity analyses reveal that the parameters of the material model and the fiber dispersion have a minor effect on the tissue response. The novel modeling approach has the potential to reduce the need of time-consuming experimental data of the mechanical behavior of fibrous tissues.

1. Introduction

Fibrous tissues are abundant constructs in blood vessels, skin, tendons, ligaments and other organs, and thus play an important role in various physiological and pathological conditions. The functionality of these tissues and the progress of diseases are affected by the mechanobiology at the ultrastructure such as in the case of abdominal aortic aneurysms. Nerem and Seliktar (2001) emphasized the effect of changes in the mechanical environment on the remodeling of the collagen network. Moreover, many methods of clinical treatments and surgical interventions rely on mechanical stimulations (Humphrey and Epstein, 2002). However, several questions concerning the interplay between the tissue functionality and the heterogeneous structure of collagen fibers are still open. From a mechanical viewpoint, the aortic media and adventitia provide the blood vessel its strength and stiffness (Taber and Humphrey, 2001). Specifically, collagen fibers are the main load bearing component in the tissue. In the media the collagen fibers are rather straight, whereas in the adventitia they admit a (more) wavy structure (Holzapfel, 2006; Schriefl et al., 2012a). Collagen fibers are embedded in a rather soft matrix, thus providing the tissue its flexibility. Taken together, from a mechanical standpoint, these are the two primary constituents composing the heterogeneous structure of the tissue.

When the tissue is not subjected to loads, the fibers are in their resting state. Once the tissue stretches the collagen fibers resist the load and, as they stretch, they stiffen the tissue. The stiffening prevents the tissue from overstretched and rupture. The relationship between the tissue microstructure and its mechanical response was investigated in various tissue types (Polzer et al., 2015; Szczesny and Elliott, 2014; Weisbecker et al., 2013). For example, the mechanical behavior of the arterial wall
was found to be mainly governed by the arrangement and the composition of collagen fibers embedded in an extra-fibrillar-matrix (Holzapfel, 2008). In recent decades mechanical constitutive models of fibrous tissues were developed to better characterize their behavior under physiological and pathological conditions (Lanir, 1983; Linka et al., 2018; Tang et al., 2009). These models belong, in general, to one of two classes: phenomenological or structural. The former can provide good estimation to measured data by appropriately fitting selected mathematical functions, but these models account indirectly for the morphological information of the tissue. At the other hand, the structural models specifically incorporate information about morphological and geometrical parameters. Phenomenological models were developed by Chuong and Fung (1983), where the macroscopic nature of the tissue is characterized by a stress-strain relationship derived from an exponential strain-energy function. This approach does not explicitly capture the mechanical behavior of the two primary constituents of the tissue, i.e., the fibers and the matrix. These models treat the tissue as a homogeneous material and frequently the constitutive parameters have no explicit physical meaning. Moreover, the constitutive parameters need to be reevaluated for each sample instead of being extracted from the available data concerning the tissues’ morphology.

Models that rely on histological/image data are becoming common in recent years (Holzapfel and Ogden, 2009). In particular, arterial layers have distributed collagen fiber orientations and they vary across the vessel walls to keep their functionality (Hariton et al., 2007; Holzapfel, 2000; Holzapfel et al., 2002). Thus, in order to model a reliable response of fibrous tissues one needs to account for the actual fiber distributions which may be based on histological/image data. Holzapfel et al. (2002) proposed a multi-layer model of the artery, where each layer in the artery is treated as a fiber-reinforced composite. Weisbecker et al. (2015) used probability-density distributions accounting for the orientation and the waviness of collagen fibers. Nonetheless, while this model accounts for the fiber distribution and waviness in terms of distribution functions, the fibers are not explicitly accounted for. In order to accurately quantify the morphology and the orientation of fibrillar tissues three-dimensional (3D) image analysis techniques were developed, which provided accurate information concerning the spatial/orientational distributions of the (collagen) fibers throughout the thickness of the tissue (Rezakhaniha et al., 2012; Schriefl et al., 2013). The recent study by Niestrawska et al. (2016) incorporated microstructural data in their modeling approach using microscopic imaging. Here, once again, different fitting parameters were required for each sample. More recently, Wang et al. (2017) acquired in vivo image data to capture the 3D geometry of blood vessels, and accounted for it in their model. Nonetheless, the material properties were defined for the complete tissue without accounting for the individual constituents.

In the above mentioned approaches there is a need to test and fit the material model of each tissue independently. This implies high costs and time consuming experiments at the one hand, and a limited ability to predict the tissue behavior on the basis of microscopy imaging at the other. It is becoming evident that simulations of the behavior of the microstructure are necessary to study the mechanical response of materials, and specifically those of biological tissues. To date, very few structurally and micromechanically-motivated models that attempt to fully capture realistic morphological and geometrical parameters were developed. For example, deBotton and Shmuel (2009, 2010) developed 3D models describing the hyperelastic behavior of fibrous tissues with one and two families of fibers, while deBotton and Oren (2013) developed a 3D periodic model for collagenous tissues capturing the fibers and the matrix structure. Their model assumes straight fibers and the fiber recruitment process is captured by using the model of Gent (1996). Fallah et al. (2016) developed a 2D model of periodic unit cells representing the heterogeneous structure of soft connective tissues based on histological and experimental data available in the literature. The discrete homogenization technique was used by El Nady and Ganghoffer (2016) to determine the overall response of 2D membranes composed of periodic fibrous networks. Subsequently, it was Berkache et al. (2017) who determined first and second gradient continuum models of 2D networks of fibrous materials, demonstrating the influences of the network density and the fibers’ tensile and bending stiffnesses on the overall response.

Modeling the tissue microstructure on the basis of its individual constituents and their spatial arrangements can lead to a more accurate characterization of the tissue behavior in comparison to a treatment of the tissue as a homogeneous and possibly anisotropic material as common in phenomenological models. Moreover, this approach can significantly reduce the need for expensive and time consuming complicated experiments as well as to improve our prediction capabilities. To achieve this goal, there is a pressing need to further develop micromechanically-based models which are able to account for the realistic structure of tissues from microscopic imaging and to provide a reliable prediction for tissue responses. This requires efficient computational methods which are able to capture the heterogeneous, anisotropic, and the highly nonlinear nature of the tissue.

In the present study a novel micromechanically-motivated model is developed with the goal to better predict the mechanical response of the aortic media. Morphological and geometrical parameters of the collagen fibers are extracted using second-harmonic generation (SHG) microscopy images in a manner similar to the one previously reported in the literature (Chen et al., 2012; Schriefl et al., 2012b). These parameters are then directly incorporated into the model, and by using advanced computational techniques the mechanical response of the medial layer is analyzed. The techniques we are using are based on concepts from homogenization theories. Thus, the macroscopic behavior of the heterogeneous materials are evaluated from the knowledge of the response of the constituting phases and their distributions. We recall that within the framework of linear elasticity there are numerous contributions that are based on these ideas. However, the pioneering works of Hill (1972) and Ogden (1974), that extended these ideas to non-linearly deforming heterogeneous materials, are crucial for the analysis of fibrous tissues. In the present study we develop a micromechanically-based model within the framework of finite elasticity to determine the mechanical response of the aortic media. By approximating the tissue microstructure in terms of a hierarchically periodic model, with distinct fiber and extra-fibrillar matrix phases, we make use of the Hill-Mandel theory in the context of periodic homogenization to predict the mechanical response of the medial layer. The motivation behind the method implemented here originates from the ability to predict the mechanical behavior of arteries in terms of morphological and geometrical parameters without destructive techniques. Once the microstructural data for the tissue is available, accurate tissue models can be determined. In turn, these models can assist in evaluating the functionality of blood vessels, and thus may assist in making decisions concerning medical treatments.

2. Theoretical background

Consider a mapping of a body transforming from one configuration to another. The position vector of a material point in the reference configuration \( \vec{\eta} \) is \( \vec{x} \) and its position vector in the current configuration \( \vec{x} \) is labeled as \( \vec{x} \). The boundary of the body in the referential and current configurations is denoted by \( \partial \vec{\eta} \) and \( \partial \vec{x} \), respectively. The deformation of the body is characterized by the deformation map \( \vec{X} \) according to \( \vec{x} = \vec{X}(\vec{X}) \). The deformation gradient

\[
\mathbf{F} = \frac{\partial \vec{x}(\vec{X})}{\partial \vec{X}}
\]

is invertible, and hence non-singular, then

\[
\mathbf{F}(\vec{X}) \equiv \det \mathbf{F}(\vec{X}) = \frac{\partial \vec{x}}{\partial \vec{X}} > 0,
\]

as described by El Nady and Ganghoffer (2016).
where \( J \), the determinant of \( F \), is equal to the ratio between the volumes of the infinitesimal material elements \( dV \) and \( dV' \) in the current and the reference configurations, respectively. If the material is incompressible, as commonly assumed for some types of soft biological tissues such as artery walls under physiological conditions, then \( J \equiv 1 \). In the sequel we restrict our attention to the class of hyperelastic materials whose constitutive relations are given in terms of a strain-energy function (SEF) \( \Psi(F) \) such that

\[
P = \frac{\partial \Psi(F)}{\partial F}.
\]

(3)

where \( P \) is the nominal or first Piola-Kirchhoff stress tensor.

For incompressible materials, because of the kinematic constraint \( J \equiv 1 \), a pressure-like term \( p \) has to be subtracted from the stress such that (Holzapfel et al., 2000)

\[
P = \frac{\partial \Psi(F)}{\partial F} - \rho F^{-T}.
\]

(4)

This pressure term can be determined from the boundary conditions imposed on the body. The Cauchy stress tensor \( \sigma \) is related to the first Piola-Kirchhoff stress tensor via the relation \( \sigma = J^{-1}PF^T \). In the absence of body forces and the acceleration, the equilibrium equation reads

\[
div \sigma = 0,
\]

(5)

where the differential operator \( div(*) \) characterizes the quantity (*) with respect to the current configuration.

Fibrous tissues such as the media are heterogeneous materials in which there is a distinct scale separation between the size of the characteristic heterogeneity (the collagen fibers) and the size of the tissue (e.g., the thickness of the layer). To effectively describe the behavior of such heterogeneous materials one needs to characterize their macroscopic behavior in terms of relevant information stemming from the microstructural level. To this end a homogenization theory for finitely deforming materials was introduced in the works of Hill (1972) and Ogden (1974). Following these we may consider the homogeneous boundary condition

\[
x = F_0 X,
\]

(6)

which is applied on the boundary of the heterogeneous body, where \( F_0 \) is a constant tensor with \( det F_0 > 0 \) (for incompressible materials \( det F_0 \equiv 1 \)). Additionally, we assume that at the interfaces, i.e. between the phases composing the material, both the displacements and tractions are continuous. Consequently, it can be shown that the average deformation gradient, say \( \bar{F} \), is

\[
\bar{F} = \frac{1}{V} \int_{\Phi_0} F(X) dV = F_0.
\]

(7)

Accordingly, the average first Piola-Kirchhoff stress tensor is

\[
\bar{P} = \frac{1}{V} \int_{\Phi_0} P(X) dV.
\]

(8)

According to the Hill-Mandel identity, when the heterogeneous body is subjected to a homogeneous incremental deformation \( x = F_0 X \), we have

\[
\frac{1}{V} \int_{\Phi_0} \bar{F} \cdot P dV = \bar{F} \cdot \bar{P}.
\]

(9)

where we note that \( \bar{F} = F_0 \). For heterogeneous hyperelastic materials this identity enables us to determine the effective SEF, i.e.

\[
\bar{\Psi}(F_0) = \inf_{F \in \mathcal{F}(\Phi_0)} \left( \frac{1}{V} \int_{\Phi_0} \Psi(F, X) dV \right),
\]

(10)

where \( \mathcal{F}(\Phi_0) \equiv \{ F(X) = \Psi(X)/\Psi(X) \in \Phi_0 \} \) is the set of admissible deformations (Hill, 1972). Note that \( \Psi(F, X) \) is the local SEF of the heterogeneous material. Specifically, consider a two-phase fibrous tissue such that \( \Psi(F) \) describes the response of the stiffer fibers and \( \Psi_0(F) \) describes the response of the softer matrix. In this case \( \Psi(F, X) = \Psi(F) \) if \( X \) corresponds to a point in the fibrous phase and \( \Psi(F, X) = \Psi_0(F) \) if \( X \) corresponds to a point in the matrix phase. According to this definition, it follows from the identity in (9) that the macroscopic constitutive behavior is

\[
\bar{P} = \frac{\partial \bar{\Psi}(F_0)}{\partial \bar{F}}.
\]

(11)

It can be further shown that the average Cauchy stress tensor \( \bar{\sigma} \) is

\[
\bar{\sigma} = J^{-1} \bar{P} \bar{F}.
\]

(12)

Following arguments similar to the ones followed at the micro-scale, if all the constituting phases are incompressible, at the macro-level we then have

\[
\bar{\sigma} = \frac{\partial \tilde{\Psi}(F_0)}{\partial \bar{F}} - \bar{p},
\]

(13)

where \( \bar{p} \) is a macroscopic pressure-like parameter.

In the sequel we assume that the microstructure of the fibrous tissue can be characterized in terms of a periodic unit cell that repeats itself. Accordingly, the macroscopic response of the heterogeneous material is determined by applying periodic boundary conditions on the faces of the unit cell. Specifically, consider a unit cell whose dimensions are \( L \) and \( B \) along the principal material directions \( x \), \( y \) and \( z \) of the periodic unit cell. The displacements of the points on the left (\( x = L/2 \)) and the right (\( x = -L/2 \)) faces of the unit cell are related via

\[
\begin{align*}
u_{x, \text{Left}}^L &- \nu_{x, \text{Right}}^L = \bar{F}_{11} - 1, \\ \nu_{y, \text{Left}}^L &- \nu_{y, \text{Right}}^L = \bar{F}_{21} - 1, \\ \nu_{z, \text{Left}}^L &- \nu_{z, \text{Right}}^L = \bar{F}_{31} - 1.
\end{align*}
\]

(14)

Similarly, the displacements of the points on the front (\( y = T/2 \)) and the back (\( y = -T/2 \)) faces of the unit cell are related via

\[
\begin{align*}
u_{x, \text{Front}}^T &- \nu_{x, \text{Back}}^T = \bar{F}_{12} T, \\ \nu_{y, \text{Front}}^T &- \nu_{y, \text{Back}}^T = \bar{F}_{22} T, \\ \nu_{z, \text{Front}}^T &- \nu_{z, \text{Back}}^T = \bar{F}_{32} T.
\end{align*}
\]

(15)

Finally, the displacements of the points on the top (\( z = B/2 \)) and the bottom (\( z = -B/2 \)) faces of the unit cell are related via

\[
\begin{align*}
u_{x, \text{Top}}^3 &- \nu_{x, \text{Bottom}}^3 = \bar{F}_{13} B, \\ \nu_{y, \text{Top}}^3 &- \nu_{y, \text{Bottom}}^3 = \bar{F}_{23} B, \\ \nu_{z, \text{Top}}^3 &- \nu_{z, \text{Bottom}}^3 = \bar{F}_{33} - 1 B.
\end{align*}
\]

(16)

We note that within the unit cell the applied periodic boundary condition results in a displacement field that is the sum of a homogeneous and a fluctuating displacement field. The average gradient of the homogeneous field is precisely \( \bar{F} - I \), while the average of the fluctuating displacement component vanishes.

In most cases the principal stretch directions of the macroscopic right Cauchy-Green tensor \( \bar{C} = F^T \bar{F} \) are not aligned with the principal axes of the unit cell, and we find it convenient to distinguish between these lab and tissue systems. In Fig. 1 the coordinate systems for the lab and the tissue, and an intermediate coordinate system are sketched together with the transformations between them. The axes are labeled according to the unit vectors along which they are directed. In particular, for the class of fibrous tissues, which is the focus of this study, the tissue’s principal material directions \( x \), \( y \) and \( z \) are represented in Fig. 1(c) and (d) by the axes \( x \), \( y \) and \( z \), respectively. The longitudinal direction along the fibers is \( y \), whereas \( x \) and \( z \) are directed along the principal symmetry axes of the plane transverse to the fiber direction. The lab coordinate system \( \bar{\theta}, \bar{Z} \) and \( \bar{R} \), which is associated with the circumferential, the axial and the radial directions of the aorta, is represented by the axes \( \theta, Z \) and \( R \) in Fig. 1(a) and (b).
The boundary conditions are imposed in the lab system in terms of the macroscopic deformation gradient, which reads in matrix form as

$$[F_{\text{lab}}] = \text{diag}[\lambda_0, \lambda_z, (\lambda_0 \lambda_z)^{-1}], \quad (17)$$

where $\lambda_0$, $\lambda_z$ and $\lambda_0 \lambda_z$ are the principal circumferential, axial and radial stretch ratios of the macroscopic right stretch tensor. For convenience, we also define an intermediate coordinate system $R, y$ and $z'$, with the axes $R$, $y$ and $z'$ such that the plane transverse to fiber is spanned by the $R$ and the $z'$ axes, see Fig. 1(c).

The deformation gradient $F_i$ induced on the unit cell is

$$F_i = Q_i Q_{\text{lab}}^T Q_i^T Q_{\text{lab}}, \quad (18)$$

where the rotation tensor $Q_i$ can be written as the orthogonal transformation matrix

$$[Q_i] = \begin{bmatrix} 0 & 0 & 1 \\ \cos \alpha & -\sin \alpha & 0 \\ \sin \alpha & \cos \alpha & 0 \end{bmatrix}, \quad (19)$$

where $\alpha$ is the angle about the radial direction $R$ between the circumferential direction $\beta$ and the fiber direction $y$ (Fig. 1(b)), and the matrix form of $Q_{i,j}$ reads

$$[Q_{i,j}] = \begin{bmatrix} 0 & \cos \beta & 0 \\ \sin \beta & 0 & \sin \beta \\ 0 & -\sin \beta & \cos \beta \end{bmatrix}, \quad (20)$$

This matrix transforms vectors from the intermediate system to the tissue’s principal material system, while $\beta$ is the angle about $y$ in the transverse plane between the radial direction $R$ and the tissue principal axis $x$, see Fig. 1(c). Finally, once the average stress $\sigma_i$ is determined by averaging the stress within the unit cell according to (8) and (12), the stress in the lab system is determined by

$$\hat{\sigma}_i = Q_i^T \sigma_i Q_{i,j} Q_{i,j}^T Q_i, \quad (21)$$

3. Experimental method

The two samples investigated in this study were acquired from Niestrawska et al. (2016). Both samples were collected as intact tubes within 24 h of death and stored in a 0.9% physiological saline solution at 4°C until testing. The use of autopsy materials was approved by the Ethics Committee of the Medical University of Graz (27–250 ex 14/15).

3.1. Microstructure

The samples were collected as intact tubes within 24 h of death and stored in a 0.9% physiological saline solution at 4°C until testing. The use of autopsy materials was approved by the Ethics Committee of the Medical University of Graz (27–250 ex 14/15). The samples were collected as intact tubes within 24 h of death and stored in a 0.9% physiological saline solution at 4°C until testing. The use of autopsy materials was approved by the Ethics Committee of the Medical University of Graz (27–250 ex 14/15).

A combination of Fourier power spectrum analysis and wedge filtering was used to extract morphological collagen data, as described in Schriefl et al. (2012b, 2013). Discrete angular distributions of relative amplitudes resembling the fiber orientation were the results of this analysis.

The fiber diameter was measured by randomly choosing 10 measuring points per image, which resulted into 700 measurements per media of each sample, by measuring them using Fiji (Schindelin et al., 2012). The density was measured utilizing the color threshold function in Fiji on each image manually. The mean values of these two parameters were used for the construction of the model described next.

3.2. Mechanics

Adjacent to the sample cut for imaging, a 20 × 20 mm patch was cut for mechanical testing. The circumferential direction was marked by removing two opposite corners. The mean thickness was measured according to Sommer et al. (2008), and a black tissue marker was applied on the surface of the samples, thus generating a scattered pattern suitable for optical tracking. Subsequently, the samples were mounted on a biaxial testing device via hooked surgical sutures, submerging them in 0.9% physiological saline solution at 37 ± 0.5°C. For testing, a stretch-driven protocol was used, starting at 2.5% deformation and increasing stepwise with 0.025 stretch until failure. Five different loading ratios were tested: $\lambda_{\text{axial}}/\lambda_{\text{circ}} = 1: 1, 0.75: 1, 1: 0.5$ and 0.5: 1, where $\lambda_{\text{axial}}$ denotes the stretch in the axial direction and $\lambda_{\text{circ}}$ is the stretch in the circumferential direction. Each increment in the stretch was followed by four preconditioning cycles and one measuring cycle, that was used for analysis. To minimize the sensitivity to initial preloads, zero strain was defined at a configuration under 0.005 N load.

4. Modeling

We designed an image-based hierarchical periodic model representing the medial tissue on the basis of its geometrical and morphological parameters, as measured by the above described image processing technique. The fiber volume fraction and the diameter were $C_i = 0.35$ and $d = 2.43 \, \mu m$, respectively. These values are approximated and may depend on the used image processing method.

In order to model the medial layer on the basis of SHG images we first obtained the number of fibers along each direction $N_i(\alpha_i)$, where $i = 1, \ldots, M$, $M$ is the number of slices, and $\alpha_i \leq \alpha_i \leq \alpha_i / 2$ denote the angles of the discrete fiber directions accounted for in the SHG images. These directions were measured relative to the circumferential direction, see $\alpha$ in Fig. 1. Second, for each angle $\alpha_i$, we averaged the number of fibers across the thickness of the media to obtain the fiber distribution function

$$N(\alpha_i) = \frac{1}{N_f} \sum_{i=1}^{M} N_i(\alpha_i), \quad N_f = \sum_{i=1}^{M} \sum_{\alpha_0}^{N_i(\alpha_i)}$$

Fig. 1. Coordinate systems involved in the modeling: (a) the lab system representing the aortic principal directions; (b) the lab and the intermediate systems sketched on an element extracted from the aorta; (c) the intermediate and the tissue’s principal material systems sketched on a layer with one family of fibers; (d) the periodic unit cell sketched with the tissue’s principal material system.
where \( \mathbf{p} \), \( \mathbf{R} \), \( \mathbf{j} \), and \( \mathbf{p} \) are defined as in (23). The histograms and the corresponding fiber distributions for the two tissue samples AA 1 and AA 2 are shown in Fig. 2, in addition to the von Mises distribution \( f(\alpha, x) \), according to

\[
f(\alpha, x) = \frac{e^{\kappa \cos \alpha}}{2\pi L_0(\kappa)},
\]

that was fitted to the data. In (24) \( L_0(\kappa) \) is the modified Bessel function of order 0. The values for the von Mises parameter \( \kappa \) are 1.852 and 1.496 for the samples AA 1 and AA 2, respectively. The heights \( n_p \) for the 6 bins are listed in Table 1.

To model the tissue we consider a two-level hierarchical approach consisting of a periodic six-layered laminate in which the individual layers are modeled as fibrous periodic structures with one family of fibers (see Fig. 1). At the upper hierarchy the fiber dispersion is accounted for by considering six families of fibers that correspond to the six layers in the periodic laminate. The relative thicknesses of the layers are determined according to the fractions of the fibers along these directions, that is \( n_p \). We find that under stretching along the principal orthotropic (circumferential and axial) directions of the tissue, a refinement of the directional distribution data to more than six angles has a negligible effect on the overall mechanical response of the tissue.

We note that at the lower hierarchy there is some arbitrariness in the choice of the details of the unit cell composing the layers, and thus choices of the cell structures that are different from the ones we assume herein are possible. In the preset model the repeating unit cell contains seven fibers in a hexagonal pattern. Four repeating unit cells are depicted in Fig. 3 versus a single layer of the media. Once the somewhat arbitrary distribution of the fibers in the transverse plane is set, the geometry of the periodic unit cell can be determined. The fiber volume fraction \( C_f \) is the ratio between the area of the fibers’ cross sections and the area of the unit cell basis. Thus,

\[
C_f = \frac{7\pi d^2}{4BL},
\]

where \( L \) and \( B \) are the dimensions of the base of the unit cell such that \( L = (1 + \sqrt{3})b, B = 3b \).

To examine the mechanical response of the tissue we chose the Yeoh SEF, say \( \Psi_Y \), for the collagen fibers and the neo-Hookean SEF \( \Psi_H \) for the matrix. These functions are given by

\[
\Psi_Y(I_1) = \sum_{i=1}^{3} C_i(I_1 - 3),
\Psi_H(I_1) = D_i(I_1 - 3),
\]

where \( C_i, i = 1, 2, 3 \), are the three Yeoh model parameters, and \( D_i \) is half

---

![Fig. 2. Fiber distribution \( \hat{N} \) vs fiber angle \( \alpha \): (a) AA 1 sample; (b) AA 2 sample.](image)

**Table 1**

| Fiber directions \( \alpha_p \) and heights \( n_p \) for the 6 bins. |
|-----------------|-----------------|-----------------|
| \( \alpha_p \) | \( \pi/12 \) | \( \pi/4 \) | \( 5\pi/12 \) |
| AA 1 sample | 0.263 | 0.127 | 0.109 |
| AA 2 sample | 0.270 | 0.142 | 0.086 |

---

![Fig. 3. (a) SHG image of a single layer of the medial layer; (b) four periodic unit cells that represent a single layer in the periodic model.](image)
is the first invariant of the right Cauchy-Green tensor \( C = F^TF \). We note that both models can be traced back to the general model of an infinite power series in the isotropic invariants, as proposed by Ogden (1997).

We make use of the Yeoh model to capture the behavior of the collagen fibers in the deformation range \( 1 \leq \lambda \leq 1.3 \). We note that a choice of an exponential model for the response of the collagen fibers is probably more natural than the Yeoh model. Nonetheless, in this limited range of deformation the behavior predicted by the Yeoh model can be approximated with an appropriate choice of the parameters of an exponential model. Yet, while the exponential model has only two fitting parameters, the Yeoh model has three, hence providing more flexibility to accurately approximate the experimental data. Moreover, the choice of the Yeoh model provides the ability to relate the parameters of the three terms with the different deformation ranges. The parameter of the first term controls the response at small deformations, whereas the third parameter becomes the dominant one at larger deformations.

We stress the fact that the transverse behavior of the unit cell, as shown in Fig. 4, is not isotropic. The transverse anisotropy of the unit cell was examined by loading the cell along different directions in the transverse plane. The stiffest direction was obtained with \( \beta = 0 \) in Eq. (20), and the softest direction was attained when \( \beta = \pi/4 \), see Fig. 1. Note that the honeycomb layout has a \( \pi/3 \) rotational symmetry, where each \( \pi/3 \) slice has a reflectional symmetry about its centerline. Thus, the behavior of the material in the transverse plane is stiffest along the angles \( \beta = \pi n/3 \) and softest along \( \beta = \pi (n/3 \pm 1/12) \), where \( n \) is an integer. An intermediate response is admitted along \( \beta = \pi (n/3 \pm 1/24) \), which is halfway between the stiffest and the softest directions. Accordingly, we choose \( \beta = 9\pi/24 \) to be the angle along the normal to the layer's plane.

To determine the response of the laminate we imposed the macroscopic deformation gradient \( F_0(\alpha_0) \) on the six layers by taking \( \alpha_0 = \pm \pi/12, \pm \pi/4 \) and \( \pm 5\pi/12 \) in (19), and determined the mean stress \( \bar{\sigma}_{\text{lam}} \) in each layer using (21). The overall stress in the laminate is then the weighted sum of the stresses developing in the individual layers (deBotton and Oren, 2013), i.e.

\[
\bar{\sigma}_{\text{lam}} = \sum_{j=1}^{6} \eta_j \bar{\sigma}_{\text{lab}}(\alpha_j).
\]

We note that for the present simulations, due to the symmetric distribution of the fibers and the biaxial loading, only the three fiber families with \( \alpha_0 = \pm \pi/12, \pm \pi/4 \) and \( 5\pi/12 \) are needed.

Biaxial stretches with the ratios 1: 1, 1: 0.75, 0.75: 1, 1: 0.5 and 0.5: 1 were imposed on the model as used for the experimental data. The parameters of the Yeoh model for the fiber phase were chosen by fitting the finite element (FE) generated stress-stretch curves in the circumferential and the axial directions to the corresponding experimental data. We note that the choice of the matrix shear modulus \( 2D_0 \) has a negligible effect on the overall response, and we set it to be two orders of magnitude smaller than the fiber material. Note that under all loading conditions the experimental stress-stretch curves for both samples are almost linear up to a stretch ratio of about 1.1, we initially determined the parameter for the linear term in the Yeoh model for the fibers of the two samples. Since the small strain responses of the two samples are quite similar, in agreement with the similarity of their fiber distributions, we found that the choice \( C_1 = 80 \text{kPa} \) provides an excellent fit for the responses of both samples under all loading conditions. Next, the other two parameters of the model for the fibers were determined by fitting the overall response of the model to the experimental data. The resulting numerical values of the parameters are summarized in Table 2. Remarkably, we note that for the quadratic term of the Yeoh model for both samples the parameter \( C_2 \) is identical, supporting the conjecture that the response of tissues can be determined from knowledge of their microstructural parameters. The material parameter \( D_1 \) of the neo-Hookean model used for the matrix phase of both samples is 0.75 kPa.

Together with the FE analysis we carry out analogous analyses that are based on a micromechanical analytical model for finitely deforming fibrous composites (deBotton and Shmuel, 2010). This model provides an extension to the micromechanical model developed in deBotton (2005), deBotton and Hariton (2006) and deBotton et al. (2006) for fibrous materials with a neo-Hookean phase. Thus, the model can be used for fibrous materials with general constitutive behaviors of the fiber and the matrix phases. Herein we use this model to determine the individual responses of the six layers in the periodic laminate. Essentially, it replaces the unit cell model in a manner similar to the one followed in deBotton and Oren (2013). Once the stresses in the layers are determined, the overall stress response of the tissue is determined by making use of (29). We stress the fact that there was no separate fitting process for the parameters of the analytical model and, in the following, the results that are shown for the analytical model are based on the same material parameters used for the numerical model.

### Table 2

<table>
<thead>
<tr>
<th>Parameter [kPa]</th>
<th>( C_1 )</th>
<th>( C_2 )</th>
<th>( C_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA 1 sample</td>
<td>80</td>
<td>250</td>
<td>3920</td>
</tr>
<tr>
<td>AA 2 sample</td>
<td>80</td>
<td>250</td>
<td>180</td>
</tr>
</tbody>
</table>

5. Results and discussion

For each biaxial test the experimental data of the samples AA 1 and AA 2 together with the corresponding results from the FE and analytical models are shown in Figs. 5 and 6, respectively. The results are illustrated in terms of the Cauchy stress versus stretch. In addition, the numerical predictions for the stress-stretch behaviors of tissues in which the fibers are 10% stiffer/softer than the fitted models are also depicted in Table 2. As can be seen, the micromechanical models are able to capture the overall responses of the tissues under different loading combinations with a fairly small set of four material parameters. At a stretch ratio of 1: 1, i.e. Fig. 5(a), the fibers do not rotate and thus immediately contribute to the overall stiffening of the tissue. When subjected to other stretch ratios the fibers rotate and stretch simultaneously. Initially, the stiffening of the fibers is small, as indicated by the low slopes up to a stretch ratio of about 1.05, see Figs. 5 and 6. As mentioned before, the measured directional distributions of the fibers and their volume fractions are almost identical for the two samples. This agrees with the very similar mechanical behaviors of the two samples in the deformation range \( 1 \leq \lambda \leq 1.1 \). At larger stretches the stiffening of the tissues becomes more evident, especially in the circumferential direction along which more fibers are directed.
The FE and analytical models admit a very good agreement with experimental data for the stretch ratios 1:1, 1:0.5, and 1:0.75, see Fig. 5(a)–(c) and Fig. 6(a)–(c). For the stretch ratios 0.5:1 and 0.75:1, for $\lambda > 1.1$, the predictions of the mechanical response for both the FE and the analytical models are somewhat stiffer in the axial direction, while a very good agreement is obtained in the circumferential direction, see Fig. 5(d),(e) and Fig. 6(d),(e). This scenario may be due to the fact that under these loading conditions the applied deformation is beyond the physiological range. This might result in local tissue damage due to the (rather) large stretches in the axial direction. At this stage our model does not take into account any damage mechanism. Thus, further studies are needed to clarify this discrepancy between the experimental data and the analytical/FE results under axial type of loading. We note that similar phenomena were observed in the study of Kao et al. (2011), where the predicted response of the model at large stretches was stiffer than the one measured in the experiment.

Fig. 5. Cauchy stress vs stretch for sample AA 1 illustrating experimental data (filled circle), analytical model (solid curve), FE model (dotted curve), and predicted tissue behavior in which the fibers are 10% stiffer/softer than the fitted model (dashed curves). The left column refers to results along the circumferential, while the right column depict results obtained from the axial direction: (a) stretch ratio 1:1; (b) 1:0.50; (c) 1:0.75; (d) 0.50:1; (e) 0.75:1. Legend within the left plots of (a) refers to all plots.
We note that for some stretch ratios the stiffening of sample AA 1 is somewhat more pronounced than that of sample AA 2, reaching higher stresses in the range $\lambda \geq 1.1$. This difference is captured by the coefficient of the third term in the Yeoh model, which is larger in the model fitted to sample AA 1. We also note that the difference between the behaviors of the two samples is not related to the distributions of the fiber directions which are almost identical in the two samples. This raise the possibility that at larger strains mechanisms such as cross-links or entanglements between the fibers may kick in and obstruct the fiber deformation. This explanation is qualitatively supported by the SHG images shown in Fig. 7, where the arrangements of collagen fibers in the two samples are presented. The rather messier distribution of the fibers in sample AA 1 can be easily distinguished from the neat and finer fiber arrangement in sample AA 2. A more quantitative measure of the fibers’ arrangement is depicted in Fig. 2, which shows the variations of the fibers’ directional distributions about the fitted von Mises distribution. We note here the distinct differences between the manners in which the fiber distributions in the two samples vary relative to the

Fig. 6. Cauchy stress vs stretch for sample AA 2. For other details of the plots see the caption of Fig. 5.
fitted distribution. Thus, while the fiber distribution in sample AA 2 agrees with the fitted curve, in AA 1 the variations of the fiber distribution about the mean are large.

Our sensitivity analyses reveal that variations in the values of the constitutive moduli have a moderate influence on the overall predicted response of the tissue. Specifically, variations of ± 10% in the values of the moduli of the fiber material lead to similar overall variations in the predicted curves. We also examined the sensitivity of the tissue response on the von Mises parameter \( \kappa \). This analysis reveals very small variations in the predicted responses due to ± 20% variations in \( \kappa \) for all the stretch ratios and for both samples. Sample results of these variations are depicted in Fig. 8.

6. Conclusions

In this study we present a novel micromechanical approach for predicting the mechanical response for fibrous tissues. Specifically, we develop a periodic six-layer laminate model, which is based on SHG imaging through the tissue thickness. The fiber orientation is accounted for by considering six families of fibers that correspond to the six layers in the periodic laminate. In turn, the individual layers are modeled as a fibrous periodic structure with one family of fibers. We find that a refinement of the directional distribution data to more than six directions has a negligible effect on the predicted response of the tissue. Analytical and numerical (FE) predictions for the tissue responses were compared to experimental data.

There is a good agreement between the analytical/numerical models with experimental data for both samples for all stretch ratios. Furthermore, in a deformation range \( 1 \leq \lambda \leq 1.1 \) the mechanical responses of the two samples are fairly similar and a single model provides accurate predictions for the experimental data for both samples. For \( \lambda > 1.1 \), however, the stiffening of the tissues become more evident, especially in the circumferential direction. In cases where the stretch in the axial direction is larger, the predictions are somewhat stiffer, while a very good agreement is obtained in the circumferential direction. This scenario may be due to the fact that under these loading conditions the applied deformations are beyond the physiological deformation range. This might result in local tissue damage. We note that the response of sample AA 1 is stiffer than that of sample AA 2, implying higher-order stiffening mechanisms which may kick in at larger strains. We assume that cross-links or entanglements between the fibers may be involved in such mechanisms. Further studies are required to clarify this issue.

Sensitivity analyses concerning variations in the parameters of the constitutive relation and the distribution dispersion parameter reveal that these variations have a minor effect on the predicted tissue responses. The micromechanical approach applied in this work can be used to predict the mechanical response of any fibrous tissue having the appropriate structural information available. This can be performed without the need for different fitting parameters for each sample, and hence also without the need for repetitive and time consuming experimental data.

Acknowledgments

This work is supported by the Ministry of Science and Technology, Israel (women in science scholarship; grant number 3-14567). In addition, the first author wish to thank EMBO, Germany (grant number ASTF 275-2016) for supporting this research by a short term travel grant. Their support is gratefully acknowledged.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmbbm.2019.04.007.

References


