

Article

Improved Mechanical Characterization of Soft Tissues Including Mounting Stretches

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Abstract: Finite element modeling has become one of the main tools necessary for understanding cardiovascular homeostasis and lesion progression. The accuracy of such simulations significantly depends on the precision of material parameters, which are obtained via the mechanical characterization process, i.e., experimental testing and material parameter estimation using the optimization process. The process of mounting specimens on the machine often introduces slight preloading to avoid sagging and to ensure perpendicular orientation with respect to the loading axes. As such, the reference configuration proposes non-zero forces at zero-state displacements. This error further extends to the material parameters' estimation where initial loading is usually manually annulled. In this work, we have developed a new computational procedure that includes prestretches during mechanical characterization. The verification of the procedure was performed on the series of simulated virtual planar biaxial experiments using the Gasser–Ogden–Holzapfel material model where the exact material parameters could be set and compared to the obtained ones. Furthermore, we have applied our procedure to the data gathered from biaxial experiments on aortic tissue and compared it with the results obtained through standard optimization procedure. The analysis has shown a significant difference between the material parameters obtained. The rate of error increases with the prestretches and decreases with an increase in maximal experimental stretches.

Keywords: mechanical characterization; mounting prestretches; arterial tissue



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1. Introduction

Understanding the behavior of soft tissues, predicting the progression of diseases, or estimating the efficiency of treatments using medical devices is often tackled with numerical simulations. For example, such simulations can be used for distinguishing mechanoadaptive from maladaptive remodeling hypertension [1], simulation of heart valves [2], studying stent oversizing on arterial response [3], and many other applications. These simulations require adequate material parameters for a reliable outcome. Material parameters are obtained through experimental testing and successive fitting procedures; this results in an optimization problem where the goal is to minimize the difference between experimentally measured and model predicted values.

Majority of soft tissues (such as arterial or heart tissue, dura mater, etc.) are well-known to be anisotropic, and thus, uniaxial tensile experiments are insufficient for obtaining full information about their in vivo mechanics. For example, the arterial wall has a highly heterogeneous structure that comprises three distinct layers: tunica intima, tunica media, and tunica adventitia [4]. Each of the three layers has a different arrangement and ratio of various constituents, among whom the elastin matrix and collagen fibers are the most important for the mechanical behavior of an arterial wall [5]. The complex microstructure governs the mechanical behavior of the arterial wall, which is considered to be anisotropic and hyperelastic with a highly non-linear stress–strain relationship [6]. The in vivo multi-axial loading state can much more closely resemble the biaxial tensile experiments [7] that

have become commonly used experimental protocols in research of soft tissue mechanics [8]. However, biaxial tensile experiments are not yet standardized, leading to various experimental setups and protocols that are reported in the literature. First of all, two distinct sample geometry groups exist: square and cruciform. When it comes to arterial tissue, the use of square specimens is much more widespread since the available size of the tissue is generally limited. Nonetheless, there are examples of studies involving biaxial testing of arterial tissue in the form of cruciform samples, e.g., [9,10]. An important aspect of the experimental setup is the choice of mounting mechanism where one can choose between hooks, rakes, and clamps (for examples, see [11–13]) along with their number, size, and distribution, which are vital for the uniformity of the loading along the sample edge. The connection between the sample and mounting mechanism is naturally the location of the stress concentration, causing inhomogeneities in the stress and strain fields [14]. According to Saint Venant's principle, strains are measured in the middle of the sample where their distribution can be considered homogeneous. The strains can be measured locally in a set having a few (usually 4 to 8) markers with a video extensometer [15] or over the full field when the Digital Image Correlation method is applied [16]. Furthermore, the experiment can be governed with force, displacement, or strain-controlled protocol.

The influence of various experimental setups on the stress and strain field, and subsequently on the material parameters obtained, has been extensively researched. Zhang et al. [17] developed a general analytical method that improves experimental stress calculation for tethered configurations. Nolan and McGarry [18] analyzed the influence of transversal forces at clamps on calculated experimental stresses and suggested using inverse finite element analysis in material characterization. A mixed numerical–experimental study by Slazansky et al. [19] compared mounting with hooks and distributed clamps. The results showed that the numbers and sizes of mounting elements should be chosen according to the sample size. It has also been demonstrated that with distributed clamps, higher experimental loads could be achieved when compared with hooks without undermining the goodness of the obtained material parameters. In a study by Fehervary et al. [20], testing conditions during a biaxial experiment with rakes were investigated with a series of FE simulations. Furthermore, a novel fitting procedure based on FE-generated correction vectors that compensate for inhomogeneities caused by rakes was presented. This study was later extended, and the proposed 'Boundary Conditions Corrected—BCC' fitting method was applied in the material characterization of aortic tissue [21]. The research described in [22] focused on a comparison of loading protocols, and the conclusion was that it did not have an effect on the mechanical response of the tissue during the biaxial experiment.

Despite significant scientific contributions from the mentioned studies, none of them considered the initial loading of the sample that occurs during the mounting. Prior to the start of the experiment, the sample is excised from arterial tissue and cleaned, and the connective tissue is removed. This unloaded state is considered as a reference configuration from a continuum mechanics point of view. A sample prepared in this way is then attached to the mounting mechanism and mounted on the testing machine. Since soft tissue is very compliant, the sample has to be slightly loaded to avoid its sagging and to ensure its flattening in the horizontal plane. Otherwise, if the sample was not positioned correctly prior to the setup of the strain-measuring system, its central region would change its vertical position (height) due to experimental loading and would thus move out of the camera focus. This would result in false strain measurements or a complete inability to record them, thereby resulting in experiment failure. Obviously, the imposed loads induce strains in the samples, and the mounted configuration differs from the reference configuration. However, it is impossible to measure mounting deformations with a calibration of a camera-based system for strain tracking is initiated from an already loaded mounted configuration. These deformations are generally neglected, while initially measured forces are usually manually annulled later on. To the best of the authors' knowledge, Linden et al. [23] conducted the sole study that addressed the described problem. The study involved a comparison of standard and BCC fitting methods with their counterparts based on a newly

proposed fitting method that integrates mounting strains (referred to as prestretches). The two unknown mounting stretches were treated as optimization parameters, together with five material parameters, in a single optimization procedure. Data utilized in the study were obtained through numerical simulations of biaxial experiments. The findings of the study demonstrated the necessity of incorporating mounting strains in the fitting method to accurately determine material parameters.

In this work, we introduce a novel fitting method that incorporates the calculation of mounting strains and their effect on material parameters. The established fitting method is complementary to the one described by Linden et al. [23], but the process of calculating material parameters and mounting strains is divided into two separate tasks, thus reducing the problem complexity in the optimization procedure. The proposed fitting method has been compared to a standard fitting method and verified on ideal data generated from the FE simulations of biaxial experiments. Using these virtual experiments, we also investigated the significance of mounting stretches on the obtained results. Furthermore, the same approach was applied to data gathered from biaxial experiments on aortic tissue.

2. Materials and Methods

2.1. Continuum Mechanics Framework

Biological tissues, such as arterial walls, can undergo large elastic deformations and are often modeled within finite strain theory as hyperelastic material. During the biaxial experiment, the strain tracking system measures deformations in the loading directions. Mapping kinematics between reference and deformed configuration is achieved using deformation gradient $\mathbf{F}$. If deformation attained during the experiment is considered homogeneous and purely biaxial without shear, $\mathbf{F}$ takes diagonal form, $\mathbf{F} = \text{diag}[\lambda_x \ \lambda_y \ \lambda_z]$, where λ_i are principal stretches in directions of Cartesian coordinate system axes, $i = x, y, z$. Principal stretches are defined as a ratio between l_i , the distance in the deformed configuration, and L_i , the distance in the reference configuration:

$$\lambda_i = \frac{l_i}{L_i} = \frac{L_i + u_i}{L_i}, \quad (1)$$

where l_i is calculated as the sum of distance in the reference configuration and u_i , displacement in the direction i . Introducing incompressibility assumption $\det \mathbf{F} = 1$, according to [24], enables calculating out-of-plane stretch, $\lambda_z = 1/(\lambda_x \lambda_y)$.

Although stresses are not directly measured during the experiment, they can be calculated from experimental forces. The stress state in reference configuration can be defined with the first Piola–Kirchhoff stress tensor $\mathbf{P}$, with the only non-zero components being the following:

$$P_{xx} = \frac{f_{xx}^{\text{exp}}}{A_{0,x}} \text{ and } P_{yy} = \frac{f_{yy}^{\text{exp}}}{A_{0,y}} \quad (2)$$

where f_{xx}^{exp} and f_{yy}^{exp} are experimentally measured forces in two loading directions and $A_{0,x}$, $A_{0,y}$ are associated load-carrying cross-section areas in the reference configuration. For arterial tissue, it is much more convenient to express experimental stresses in the deformed configuration with Cauchy stress tensor $\boldsymbol{\sigma}^{\text{exp}}$ which is derived from $\mathbf{P}$ with the following expression:

$$\boldsymbol{\sigma}^{\text{exp}} = J^{-1} \mathbf{P} \mathbf{F}^T, \quad (3)$$

where J is Jacobian or volume change. Note that $J = 1$ due to incompressibility assumption.

Modeling mechanical response of hyperelastic materials is performed with strain energy density function W . The associated Cauchy stress tensor is given in the following form:

$$\boldsymbol{\sigma}^{\text{mod}} = -p \mathbf{I} + 2 \mathbf{F} \frac{\partial W}{\partial \mathbf{C}} \mathbf{F}^T, \quad (4)$$

where $\mathbf{I}$ is the identity tensor, $\mathbf{C}$ is the right Cauchy–Green deformation tensor defined as $\mathbf{C} = \mathbf{F}^T \mathbf{F}$ and p is the Lagrange multiplier related to the incompressibility assumption. Samples used in biaxial testing are thin sheets with thicknesses much lower than their length and width. Therefore, the plane stress state can be assumed with a boundary condition $\sigma_{zz}^{\text{mod}} = 0$ that allows for the calculation of the Lagrange multiplier p .

The constitutive model that is specifically developed to describe the relationship between stress and strain of arterial tissue by Gasser, Ogden, and Holzapfel [25] is further referred to as the GOH model. The structure of arterial tissue governed the definition of the GOH model, where tissue is represented as a combination of isotropic elastin matrix with two embedded symmetrically oriented collagen fiber families responsible for anisotropic behavior [26]. GOH constitutive model defines strain energy density function in the following form:

$$W = C_1 \cdot (I_{1,\mathbf{C}} - 3) + \frac{k_1}{2k_2} \sum_{i=4,6} \left\{ \exp \left[k_2 [\kappa I_{1,\mathbf{C}} + (1 - 3\kappa) I_i - 1]^2 \right] - 1 \right\} \quad (5)$$

The first part of the strain energy density function represents an isotropic elastin matrix with C_1 being a positive, shear-like material parameter and $I_{1,\mathbf{C}} = \text{tr}(\mathbf{C}) = \lambda_x^2 + \lambda_y^2 + \lambda_z^2$ being the first invariant of $\mathbf{C}$. The second part of the strain energy density function describes the contribution of two fiber families with the same material properties. The stiffness of fiber families is represented with a material parameter k_1 that ought to be greater than zero. Non-linearity of stretch–stress response is modeled with dimensionless material parameter k_2 that also must be positive. I_i are pseudoinvariants that represent a component of stretch in the mean fiber family direction projected on loading axes and are calculated as $I_i = \mathbf{M}_i \cdot \mathbf{C} \mathbf{M}_i$, where $i = 4, 6$ for two fiber families. The vector $\mathbf{M}_i^T = [\cos \alpha_i \quad \sin \alpha_i \quad 0]$ denotes mean fiber family orientation in the reference configuration. The angle α_i is a mean angle of fiber family with respect to the x direction that corresponds to the circumferential direction of the artery. Dispersion of fibers inside fiber family around mean fiber direction is modeled with parameter κ . This parameter can vary from $\kappa = 0$, in case of perfectly aligned fibers, and $\kappa = 1/3$ in case of fully dispersed fibers showing isotropic behavior. To summarize, material behavior depends on three material parameters C_1, k_1, k_2 and two structural parameters α_i and κ that have to be determined through mechanical characterization. A more comprehensive description of the presented framework can be found [27].

2.2. Material Parameter Identification Procedures

2.2.1. Standard Fitting Methods

The common approach in material parameter identification is to iteratively search for material parameters that will minimize the sum of squared differences between experimental and modeled values by means of non-linear least squares regression. When it comes to the biaxial tensile experiment, the objective function in this minimization process is based on stresses in both loading directions and takes the following form:

$$\min_{\mathbf{P}} \sum_{j=1}^n \left[\left(\sigma_{x,j}^{\text{exp}}(\mathbf{F}) - \sigma_{x,j}^{\text{mod}}(\mathbf{F}, \mathbf{P}) \right)^2 + \left(\sigma_{y,j}^{\text{exp}}(\mathbf{F}) - \sigma_{y,j}^{\text{mod}}(\mathbf{F}, \mathbf{P}) \right)^2 \right] \quad (6)$$

where j denotes the time point at which data are measured, n is the total number of time points, and $\mathbf{P}$ is a vector of material parameters that need to be determined. Two choices can be made while preparing experimentally measured force data for the calculation of experimental stresses. The first is to leave data intact, resulting in non-zero forces and zero strain state at timestep $j = 1$. This is incompatible with modeled stress since zero stress state must be associated with zero strain state. Consequently, material parameters obtained with this approach overestimate the stiffness of the tissue. The second option tries to overcome the given problem by nullifying the data, i.e., by manually subtracting forces measured at timestep $j = 1$ from force values at all timesteps $j = 1, \dots, n$. With this

artificial adjustment, a zero stress and zero strain state are achieved. However, neither of these options corresponds to real stress and strain states in mounted configuration.

2.2.2. Fitting Including Prestretch Method

In order to improve mechanical characterization, we have developed a new method that will include mounting prestretches. For this purpose, kinematics was adjusted to resemble reality more closely. As shown in Figure 1, the material testing procedure starts with an unloaded sample in reference configuration. Upon mounting the sample onto testing apparatus, it is loaded to ensure its positioning, leading to introduction of a new mounted configuration described with mounting prestretches that, unfortunately, cannot be measured. In the standard fitting procedure, mounted configuration is deemed as reference configuration with zero strain state and mounting forces that are usually annulled manually. The mounted configuration can be related to the reference configuration with newly introduced mounting deformation gradient $\mathbf{F}^m$. During the execution of the experiment, displacements in regard to mounted configuration are recorded for each time step, and experimental deformation gradients $\mathbf{F}^{\text{exp}}$ are derived. The total deformation gradient is, thus, defined as $\mathbf{F}^{\text{tot}} = \mathbf{F}^{\text{exp}} \mathbf{F}^m$. Subsequently, the right Cauchy–Green tensor is updated to $\mathbf{C} = (\mathbf{F}^{\text{tot}})^T \mathbf{F}^{\text{tot}}$.

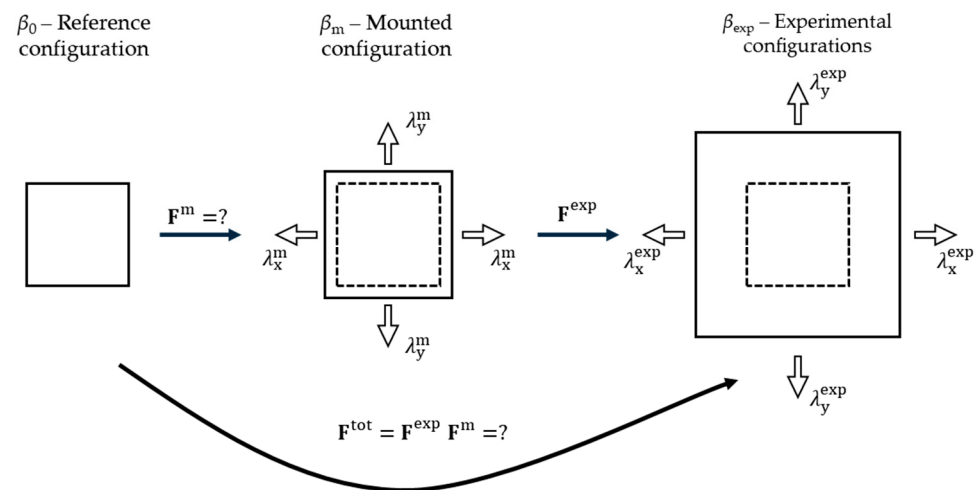


Figure 1. Biaxial experiment procedure: the unloaded sample (reference configuration), sample mounted on the machine and stretched in both directions in the process (mounted configuration, deformation gradient tensor unknown), and experimentally tested sample (experimental configurations) with corresponding deformation gradient tensors.

A new kinematics description with a mounted configuration introduces two additional unknown parameters—prestretches λ_x^m and λ_y^m . Obviously, with unknown mounting prestretches, the total deformation gradient cannot be determined. Since both experimental and modeled stresses are functions of the total deformation gradient, the proposed fitting procedure has been divided into two steps. The first step is to calculate prestretches from the difference between experimental and modeled stresses at the beginning of the experiment for the current set of material parameters. Using the calculated prestretches, the total deformation gradient can be calculated. Therefore, in the second step, a new set of material parameters can be determined through the optimization process. The flowchart of a proposed fitting method is presented in Figure 2.

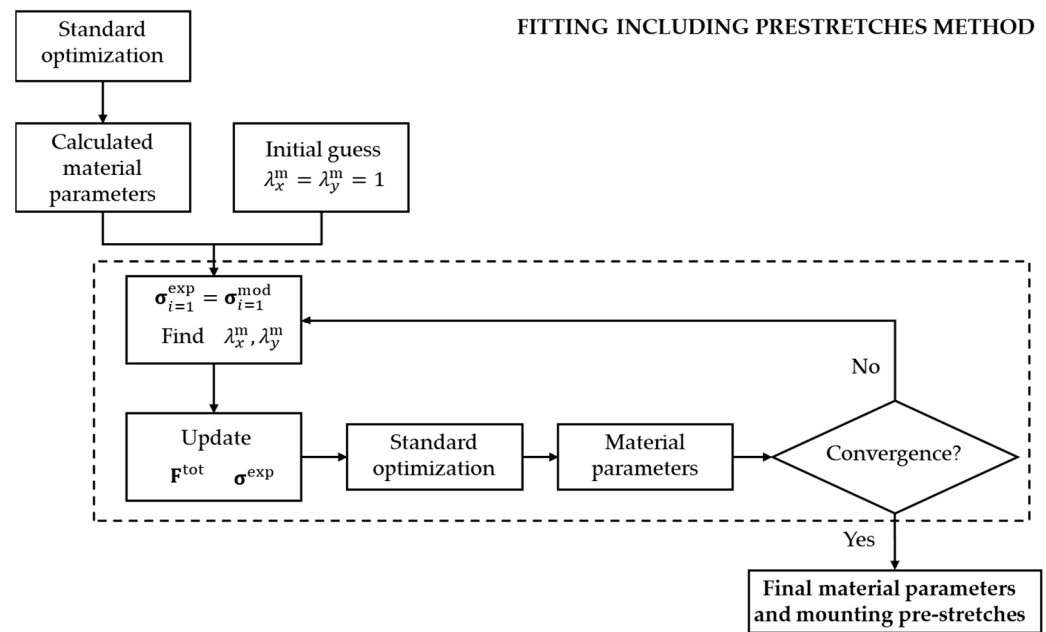


Figure 2. Flowchart of proposed fitting including prestretch method.

Initially, the mounting stretches are set to $\lambda_x^m = \lambda_y^m = 1$. In order to obtain the initial guesses for material parameters, the algorithm starts with a standard optimization procedure. Note that this step can be omitted, and any arbitrary value can be chosen for the initial guess; however, for inadequately chosen initial guesses, more iterations might be necessary for the convergence of the results. Using the assumed values of material parameters, it is possible to calculate the mounting stretches from a difference between experimental and model stresses. This comes down to a root finding problem that can easily be solved numerically, where the goal is to find the roots of a system of equations at time step $t = 0$:

$$\sigma_k^{\text{exp}}(f_k, \mathbf{F}^{\text{tot}}) - \sigma_k^{\text{mod}}(\mathbf{P}, \mathbf{F}^{\text{tot}}) = 0 \quad (7)$$

where $k = x, y$ denotes loading directions. In the above equation, the f_k are experimental forces at the beginning of the experiment (i.e., mounting forces that are usually annulled), and $\mathbf{P}$ is a set of material parameters from standard optimization or previous iteration. The root finding problem is solved with the roots function and the hybrid method from the well-known Python library SciPy. With determined prestretches, total deformation gradient and experimental stresses are recalculated. Updated experimental data are used as input to the optimization procedure. A new set of material parameters is obtained by optimization procedure for an objective function described in Equation (6). In this work, optimization process was performed with Python using curve_fit function from SciPy library, with trust-region reflective algorithm. To confirm whether the convergence is reached, two criteria need to be satisfied: first, the difference between experimental and modeled stress should be less than the predetermined tolerance, and second, the difference between material parameters obtained in the current and previous algorithm iteration should be sufficiently small. If any of the criteria is met, the algorithm is considered converged. Otherwise, subsequent iteration starts with the calculation of prestretches using the new set of material parameters.

2.3. Virtual Biaxial Experiments

In order to verify the proposed fitting method with included prestretches and to compare it with standard fitting methods, a series of FE-simulated virtual biaxial experiments has been run. FE simulations of biaxial experiments are made with Abaqus-Standard 6.14 software (Dassault Systemes) to generate experimental data, i.e., force–stretch curves, for a set of prescribed ground-truth material parameters. Furthermore, by running virtual

experiments, it is possible to avoid all measuring and other uncertainties that occur during experiments. The specimen dimensions were chosen according to real biaxial experiments, described in Section 2.4, made on the human aorta. The FE model was made with a mesh of $20 \times 20 \times 1$ C3D8H elements based on hybrid formulations that are suitable for the simulation of incompressible hyperelastic materials. The loading was applied in the form of equibiaxial displacements at specimen edges, allowing for the simple control of achieved stretches. More importantly, the loading was divided into two steps, according to Figure 1, thus separating mounting forces and prestretches from experimental forces and their associated stretches. Multiple cases have been run, varying the value of prestretches from 0.5% ($\lambda^m = 1.005$) with a stepwise increase of 0.5% up to 2% ($\lambda^m = 1.02$) while holding the total stretches at 20% ($\lambda^{\text{tot}} = 1.2$). The choice of total stretches was made according to experiments made on human aorta [28]. Two sets of ground-truth material parameters were utilized in simulations. The first set of material parameters was taken from a study [29] where they were obtained by a non-invasive fitting approach based on in vivo measurements of pressure and diameter for the human thoracic aorta. In the in vivo state, the arterial wall is primarily loaded with internal blood pressure and axial forces resulting from a stretch that is the result of growth. In healthy human arteries, the elastin matrix is a load-bearing constituent for blood pressures in the narrow physiological range (80–120 mmHg), and the stress–strain response is nearly linear [30]. Since these material parameters were calculated from the data gathered in the physiological range, this set of material parameters showed a mostly linear stress–strain response. The second set of material parameters is taken from the study presented by Weisbecker et al. [31]. Here, the material parameters are determined from biaxial experiments on human abdominal and thoracic aortas. Experimental testing allowed applying supra-physiological loading; thus, stress–strain curves show stiffening behavior typical for arterial tissue at higher stretches. The two sets of parameters are listed in Table 1.

Table 1. Sets of material parameters from the literature chosen as a ground truth for virtual biaxial experiments.

MP Set	C_1 [kPa]	k_1 [kPa]	k_2 [-]	α [°]	κ [-]
Smoljkić M55	59.7	36.3	11.38	58.9	0.243
Weisbecker—media	14.0	140	11.90	38.4	0.210

As shown in Figure 3, the force–stretch data extracted from the simulations were adjusted to mimic the realistic experimental data. During the real experiment, the sample is loaded in the mounting process (red markers in Figure 3) and then further experimentally stretched (blue markers in Figure 3). However, since mounting prestretches cannot be measured, red markers are removed from the simulated data, and the blue markers are shifted to the left (as initial stretch at timestep $t = 0$ must satisfy $\lambda_x^{\text{exp}} = \lambda_y^{\text{exp}} = 1$). We hereby generated a new artificial dataset from virtual experiments (yellow markers) that mimics the data measured in real experiments.

The simulations were made with a hundred equal increments per step, providing a hundred data points from the experimental step for data fitting. The prepared data were used as an input for three fitting methods, namely the standard fitting method, the standard fitting method with subtraction of initial forces, and the newly proposed fitting, including prestretch method. Sets of material parameters obtained with all three methods were compared to the ground-truth material parameters with the mean absolute error (MAE) defined as follows:

$$MAE = \frac{1}{n} \sum_{i=1}^{n=5} \left| \frac{P_i^{\text{GT}} - P_i}{P_i^{\text{GT}}} \right| \quad (8)$$

where $n = 1, \dots, 5$ is the number of material parameters, P_i^{GT} is the prescribed ground-truth material parameter i and P_i is the material parameter i obtained with a given fitting method. The obtained material parameters and simulated stretches were used to calculate modeled

stresses according to Equation (4). The goodness of the fit between modeled stresses and ground-truth ones was also assessed with the coefficient of determination R^2 .

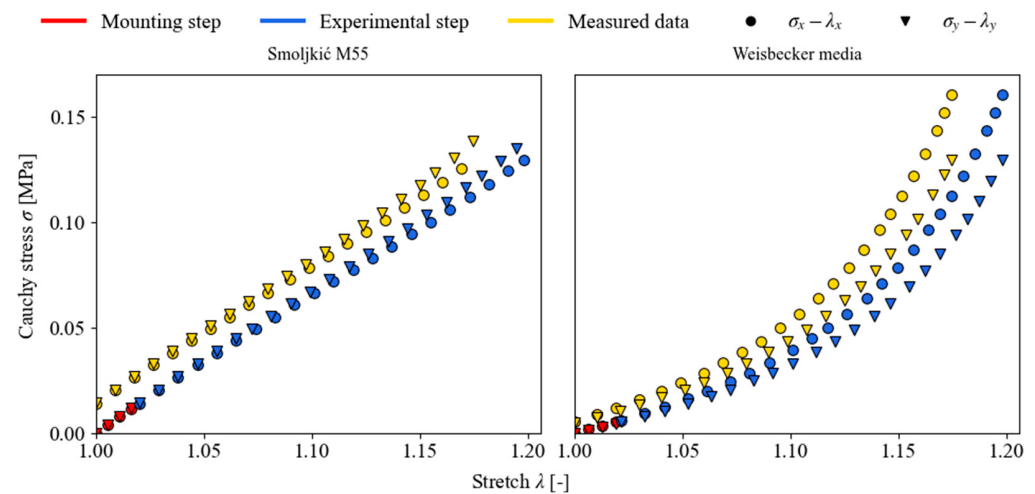


Figure 3. Comparison of ground-truth data obtained from mounting (red markers) and experimental steps (blue markers) and measured/prepared data for virtual experiments (yellow markers) on both material parameter sets with prestretch equal to 2% (material parameters from [29] on the left, and from [31] on the right).

2.4. Biaxial Experimental Testing of Human Aortas

The experimental data analyzed in this work were gathered as part of another study [28]; thus, the specimen preparation and experimental testing will only be briefly described. Human aortic samples were collected at the Institute of Pathology, Medical University of Graz. The initial step of the sample preparation included the removal of surrounding tissue and fat from the outer side of the aortas. Then, the tube-like shaped aortas were opened with a longitudinal cut. Biaxial specimens were cut out from the aorta with the help of a stamping tool, with special attention paid to the orientation so that the circumferential direction aligns with the x -testing direction while the axial direction aligns with the y -testing direction. The use of a stamping tool allowed the excision of square specimens with an edge length of 20×20 mm. During the entire time of the preparation process, samples were regularly sprayed with phosphate-buffered saline solution with a pH value of approximately 7.4. The thicknesses of specimens were measured optically with the WinextNG software package (ZwickRoell, Fuerstenfeld, Austria). An additional specimen of Donor II was stamped out and separated into three arterial wall layers. Unfortunately, the thickness of the separated layers was not measured and will thus be approximated according to [32], with the following thickness percentages—intima 16%, media 52%, and adventitia 32%—of total thickness that was 1.69 mm. Table 2 gives general information about the two donors and other important information about the experiments conducted.

Table 2. Basic information about human aorta sample donors and specimens that were experimentally tested.

Specimen	Gender	Age	Atherosclerosis Level	Thickness [mm]	Experimental Stretches [-]
Donor 1	Male	56	Medium	1.93	1.15
Donor 2	Male	69	High	1.35	1.15
Donor 2—intima	Male	69	-	0.27	1.05
Donor 2—media	Male	69	-	0.88	1.20
Donor 2—adventitia	Male	69	-	0.54	1.15

Experimental testing was performed at the Institute of Biomechanics at Graz University of Technology on a custom-built biaxial testing machine. The experimental machine operates with four independent linear actuators, each equipped with a load cell that can achieve a maximum force of 100 N and has a resolution of 0.6 mN. Strain measuring was conducted with a video extensometer (VE) that comprised the CCD camera with 0.15 μm resolution and the 'Laser Speckle Extensometer' software (ZwickRoell, Fuerstenfeld, Austria). A comprehensive description of the biaxial testing system can be found in [12,33].

Specimens were pierced with five hooks per edge and mounted on the biaxial testing machine, as shown in Figure 4. Equal distance and positioning of the hooks were ensured with a hooking template. After the mounting, specimens were loaded with forces necessary for avoiding their bending. These mounting forces were approx. 0.015 N to 0.02 N. Naturally, these mounting forces altered the strain state. The change in the strain state was not registered since a calibration of the VE can only be conducted after the positioning of the specimen. Figure 4b shows a mounted specimen prior to setup and calibration of the strain tracking system.

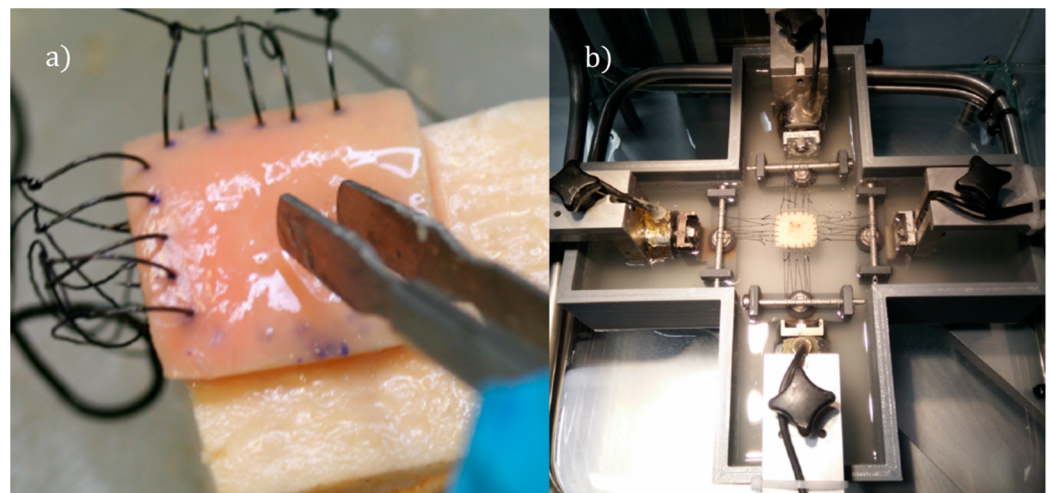


Figure 4. (a) Placement of the hooks on the specimen. (b) Specimen after mounting on the biaxial machine and prior to calibration of the VE, taken from [28] with the permission of the author.

Histological powdered dye was used for specimen dusting to create a recognizable pattern that can be tracked by VE. Experiments were performed in phosphate-buffered saline solution heated to 37 °C to mimic physiological conditions. Camera focus during the testing was ensured by placing a petri dish over the sample. The experiments were conducted in a strain-controlled protocol with the equibiaxial stretch ratio. Prior to experimental testing, all specimens were preconditioned for five cycles up to the maximum experimental stretch with a loading speed of 3 mm/min. The sixth cycle was the experimental cycle that was used for the analyses. The maximum achieved experimental stretch was defined as the stretch level where the specimen was able to withstand all six testing cycles without any sign of tissue rupture due to the hooks. The maximum stretch varied between specimens depending on the health state of the aortic tissue. Figure 5 shows the experimental Cauchy stretch–stress curves for all tested specimens. Specimens from two donors were tested, and for one of the donors, the layers of the aortas were also tested separately. Note that the specimen for Donor 1 had a much higher thickness, so the Cauchy stress values were much lower compared to Donor 2.

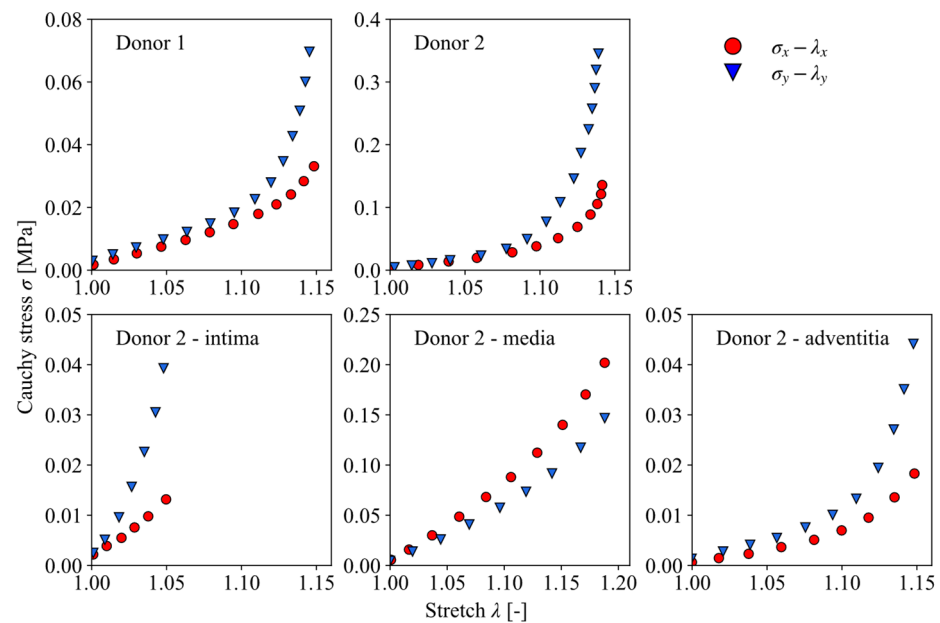


Figure 5. Experimental Cauchy stretch–stress behavior for all analyzed experiments on samples from two donors; upper figures are plotted for the whole wall, while the lower three curves reveal the layer-specific behavior of Donor 2.

3. Results

3.1. Comparison of Fitting Methods and Verification of Fitting with Prestretch Method

A total of eight datasets were generated through virtual experiments utilizing four levels of mounting stretches for two sets of ground-truth material parameters. These datasets were analyzed using three different fitting methods: standard fitting, standard fitting with the subtraction of initial forces, and fitting including prestretches. For each virtual experiment, analyses conducted with standard fitting methods were rerun five times, each time with a randomly chosen initial guess of the material parameters set. The results of analyses always converged to the same solution, making standard fitting methods independent of the initial guess. Fitting, including the prestretch method, was also applied five times per virtual experiment. In the first case, the initial guess was a solution of the standard fitting method, and four additional cases were run with randomly chosen values for material parameters. Again, all cases converged to one unique solution.

Even for the smallest level of prestretches of just 0.5%, the results of the standard fitting without subtraction of initial forces are not close to the ground-truth material parameters (shown in Tables 3 and 4). The increase in prestretches led to an increase in elastin matrix stiffness, as indicated by the material parameter C_1 . On the other hand, there is no general pattern of change in estimated values of material parameters k_1 and k_2 for either method. No clear rules were observed for the behavior of structural parameters (α and κ) in the case of Smoljkić M55 material. One of the structural parameters had the boundary value (note that α can vary between 0° and 90° , while κ is within range $[0, 1/3]$ as aforementioned) irrelevantly of prestretch level. It is noticeable that for the highest level of prestretch, $k_2 = 0$, suggesting there is no stiffening effect (see Figure 6 at 2% prestretches). Subtraction of the initial forces prior to standard fitting highly improved the accuracy of the obtained material parameter sets, as can be seen from calculated errors. Elastin matrix stiffness is underestimated for Smoljkić M55 material and is even further decreasing with the increase in prestretches. Nevertheless, the contribution of the elastin matrix to total material stiffness was compensated by an overestimation of collagen fiber family material parameters k_1 and k_2 that were increasing with increased prestretch values. The most noteworthy improvement caused by the subtraction of initial forces is noticed for structural parameters that have realistic values at all prestretch levels.

Table 3. Estimated material parameters and corresponding errors obtained using standard fitting method with and without subtraction of initial forces and fitting, including prestretch method from virtual experiments for Smoljkić M55 material at four different prestretch levels.

Fitting Method	λ_x^m, λ_y^m	C_1 [kPa]	k_1 [kPa]	k_2 [-]	α [°]	κ [-]	MAE [10 ⁻³]	R^2
Standard fitting	1.005	64.9	2.47	8.12	56.9	0	467.9	0.993
	1.010	69.3	1.62	16.3	90.0	0.1	533.0	0.970
	1.015	71.9	2.6	1.23	90.0	0	710.5	0.879
	1.020	74.6	2.89	0	90.0	0	739.6	0.868
Standard fitting w. subtraction	1.005	58.7	39.9	12.06	59.0	0.246	37.9	0.999
	1.010	57.7	43.7	12.77	59.1	0.248	76.7	0.998
	1.015	56.7	47.7	13.54	59.2	0.250	117.6	0.997
	1.020	55.8	51.9	14.36	59.3	0.251	159.3	0.995
Fitting inc. prestretch	Any value	59.7	36.3	11.38	58.9	0.243	0	1
Ground-truth		59.7	36.3	11.38	58.9	0.243	-	-

Table 4. Estimated material parameters and corresponding errors obtained using standard fitting method with and without subtraction of initial forces from virtual experiments for Weisbecker media material at four different prestretch levels.

Fitting Method	λ_x^m, λ_y^m	C_1 [kPa]	k_1 [kPa]	k_2 [-]	α [°]	κ [-]	MAE [10 ⁻³]	R^2
Standard fitting	1.005	10.3	43.9	4.93	41.45	0	523.2	0.981
	1.010	14.3	41.1	5.57	41.16	0	466.3	0.916
	1.015	18.4	38.3	6.30	40.84	0	515.0	0.781
	1.020	22.7	35.6	7.12	40.48	0	564.6	0.537
Standard fitting w. subtraction	1.005	14.0	147.9	12.49	38.47	0.209	22.5	0.987
	1.010	14.6	157.6	13.21	38.49	0.209	57.2	0.932
	1.015	14.2	169.4	14.07	38.48	0.211	82.7	0.852
	1.020	14.4	183.7	15.11	38.41	0.214	125.9	0.701
Fitting inc. prestretch	Any value	14.0	140	11.90	38.4	0.210	0	1
Ground-truth		14.0	140	11.90	38.4	0.210	-	-

The use of fitting, including the prestretch method, successfully obtained ground-truth material parameters together with corresponding prestretches for all simulated experiments. Figure 6 shows a comparison of modeled stresses calculated from ground-truth experimental stretches and sets of material parameters obtained with three fitting methods for Smoljkić M55 material. It is clearly visible that the new method perfectly coincides with the virtual experiment, whereas the standard fitting method without initial force subtraction introduces significant error even for the smallest mounting stretches. This error only increases with prestretches. Subtraction of the forces for this almost linear behavior significantly improves the accuracy of predicted curves; however, for non-linear mechanical behavior, this will not suffice, as can be seen from the material parameter set taken from Weisbecker et al. [31].

Summarized results of fitting from virtual experiments for Weisbecker media material with both standard fitting methods are shown in Table 4. Obviously, the increase in non-linearity of mechanical behavior results in a significantly reduced accuracy of parameter estimation with an increase in mounting stretches. It can be seen that at 2% prestretch, goodness of fit (R^2) amounts only to 0.537 for standard fitting without force annulment and 0.7 with it, both of which are very unsatisfactory. Even for the lowest prestretch level, large errors occur.

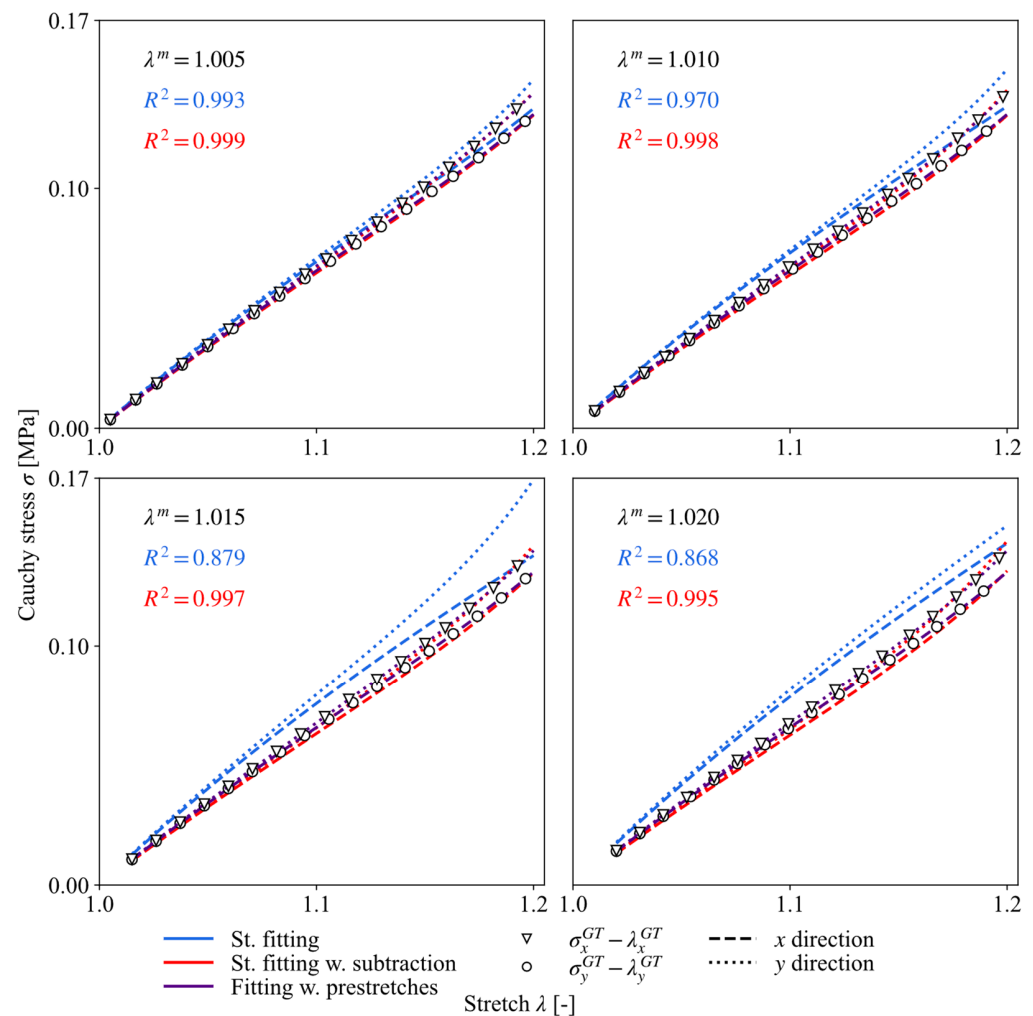


Figure 6. Comparison of modeled stresses with ground-truth data for sets of material parameters obtained with three fitting methods for four simulated virtual experiments with Smoljkić M55 material.

Similarly, for the conclusions drawn for the Smoljkić M55 material, standard fitting method results show that an increase in prestretches increases elastin matrix stiffness as described with C_1 . An inverse relationship of material parameters k_1 and k_2 is exhibited. The increase in prestretches resulted in reduced stiffness of the collagen fiber family, expressed with k_1 , which is compensated by an increase in the stiffening effect denoted by k_2 . However, both parameters are underestimated when compared to the ground-truth ones. Regarding structural parameters, α remained relatively constant while κ was consistently zero, which indicates that there is no dispersion of fibers around their mean direction. On the other hand, standard fitting with subtraction of initial forces always resulted in realistic (non-boundary) values of the structural parameters.

A noteworthy overestimation of collagen fiber family material parameters k_1 and k_2 is observed for results of standard fitting with subtraction of initial forces method. Therefore, significant overestimation of modeled and ground-truth stresses happens at higher stretches. Material parameters k_1 and k_2 are increasing with prestretch values; thus, specified behavior is most notable at prestretches of 1.5% and 2% (see Figure 7). This is expressed in an evident drop of R^2 values at specified prestretch levels, unlike Smoljkić M55 material, where R^2 was higher than 0.99. Again, using fitting with the prestretch method, ground-truth sets of material parameters have been obtained at all prestretch levels. Since ground-truth and obtained material parameters have been the same, the related R^2 value is practically equal to one. Applying the proposed fitting, including the prestretch method on ideal data from virtual experiments, ensured its verification.

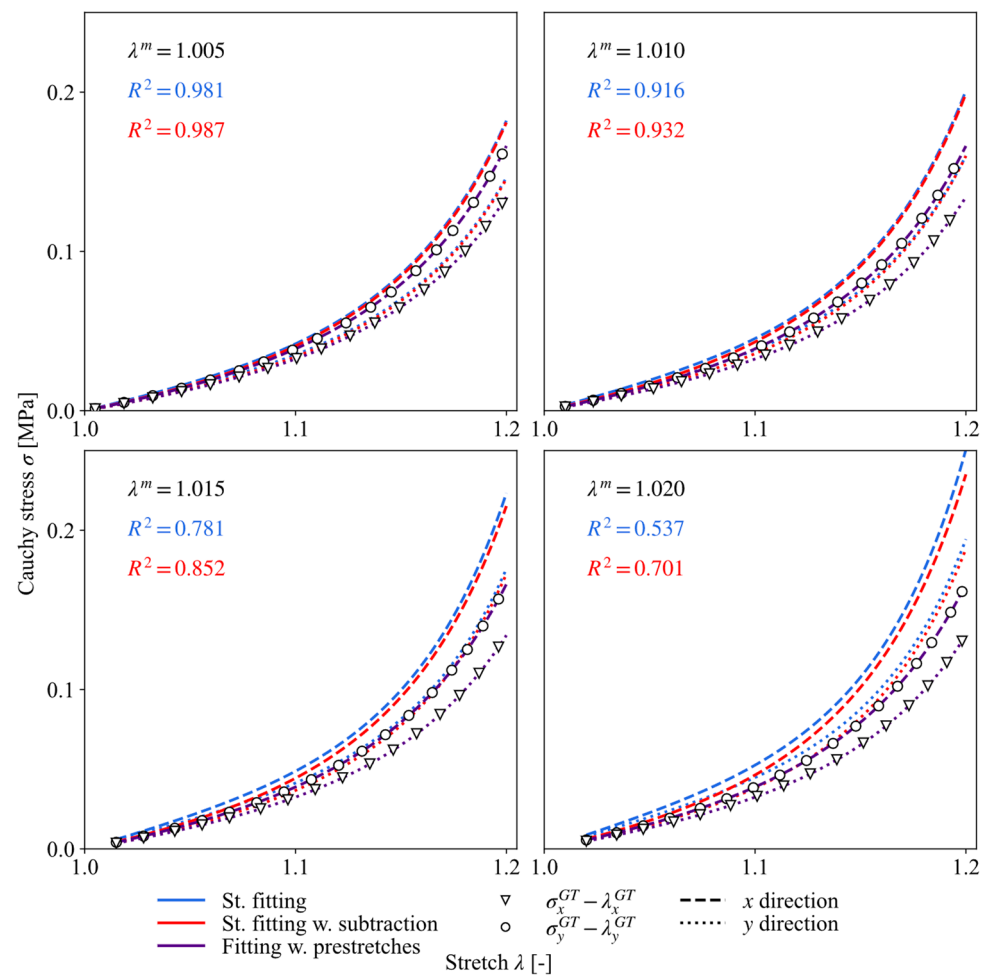


Figure 7. Comparison of modeled stresses calculated from ground-truth experimental stretches and sets of material parameters obtained with three fitting methods for Weisbecker media material.

Modeled stresses based on ground-truth stretches and sets of material parameters obtained by three fitting methods are compared to the ground-truth stresses for Weisbecker media material, as shown in Figure 7. Clearly, neglecting the mounting stretches overestimates the overall stiffness of the arterial wall.

From the results in Tables 3 and 4, it is noticeable that the MAE for sets of material parameters obtained with subtraction of initial forces is lower by an order of magnitude than the MAE for sets of material parameters obtained with standard fitting. Furthermore, it seems the MAE behaves almost as a linear function of prestretch for standard fitting with subtraction of initial forces (see Figure 8). However, this was not the case for standard fitting, probably due to various combinations of obtained material parameters having boundary values at different prestretch levels. The increase in prestretches caused the drop of R^2 , regardless of the standard fitting method choice. The calculated MAE and R^2 as functions of prestretch for both materials and two standard fitting methods are shown in Figure 8.

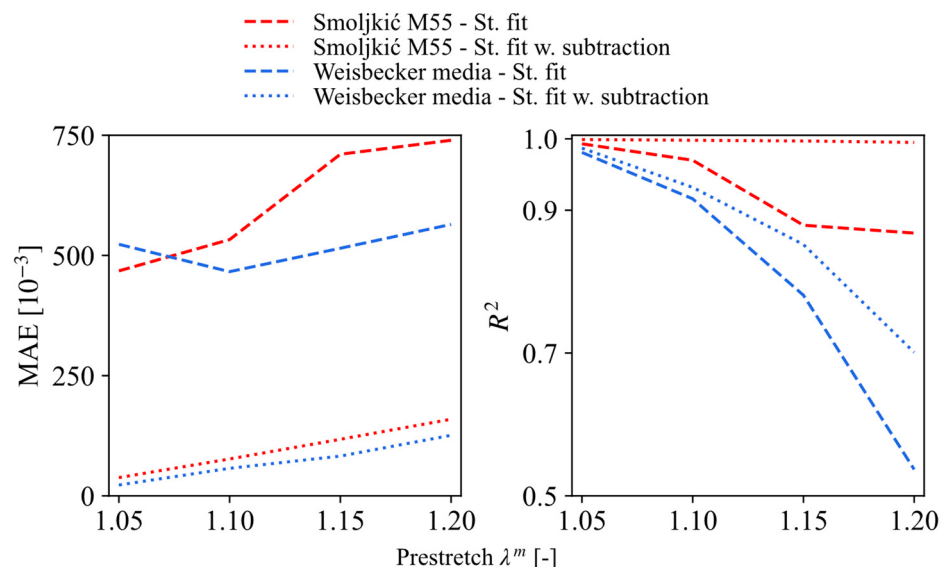


Figure 8. Influence of prestretch on goodness of fit obtained with two standard fitting methods applied on data from virtual experiments expressed with mean absolute error MAE (left) and R^2 (right).

Note that standard fitting methods resulted in high goodness of fit (with $R^2 \approx 0.99$) when compared to the measured data (yellow markers in Figure 3). This means that the selected material model and optimization algorithm (Trust region reflective) are appropriate. However, the measured data do not represent the ground-truth data (denoted by blue markers in Figure 3). Modeling stresses using the obtained material parameters and ground-truth stretches results in significantly lowered coefficients of determination. These R^2 values are summarized in Tables 3 and 4.

The fitting, including the prestretch method, has been successful in overcoming the described problem. Iterative calculation of prestretches enables the updating of measured data and results in obtained sets of material parameters equal to the ground-truth ones. In this way, a newly proposed fitting method has been validated. It is also worth noting that the fitting method, including prestretches, required about 10–20 iterations in each case, making it computationally inexpensive. The low number of iterations is attributed to the absence of measurement uncertainties in virtual experiments and to the usage of the same material model (GOH) for fitting as in virtual experiments.

3.2. Fitting Including Prestretch Method—Comparison with ‘Linden Method’

Further validation of the fitting method, including prestretch, was made on experimental data reported in a study by Linden et al. [34] with publicly available raw data from biaxial experiments together with material parameters and prestretches calculated with previously referenced the ‘Linden method’ presented in [23]. Five ovine thoracic aorta samples were biaxially tested and raw data measured with 2D DIC are analyzed herein. The results reported in [34] and sets of material parameters and prestretches calculated with the fitting, including the prestretch method, are summarized in Table 5. The sets of material parameters obtained with both methods provided high-quality fits for their experimentally updated data. This is exhibited in terms of R^2 , which was greater than 0.99 in all examples. Figure 9 shows a comparison of stretch–stress curves modeled with the ‘Linden method’ and the fitting, including the prestretch method with the corresponding updated experimental data. As can be seen from the picture, there is basically an ideal overlap of curves for specimens 1, 2, and 4. Note that in the Figure 9 legend, LM stands for the ‘Linden method’ and FIPM for fitting including the prestretch method.

Table 5. Sets of material parameters and affiliated prestretches calculated with ‘Linden method’ and the fitting, including prestretch method for data obtained from biaxial experiments on ovine thoracic aortic samples. Note that FIP stands for fitting, including prestretch method.

Specimen	Method	C_1 [kPa]	k_1 [kPa]	k_2 [-]	α [°]	κ [-]	λ_x^p [-]	λ_y^p [-]
1	Linden	5.1	20.2	2.110	89.98	0.263	1.077	1.058
	FIP	5.1	20.1	2.154	85.76	0.263	1.077	1.059
2	Linden	8.0	43.5	1.057	90.00	0.295	1.063	1.042
	FIP	8.0	44.0	1.138	90.00	0.298	1.060	1.044
3	Linden	1.0	18.6	0.029	46.86	0.059	1.066	1.043
	FIP	2.4	21.3	0.039	46.60	0.120	1.056	1.052
4	Linden	6.8	41.0	0.322	90.00	0.277	1.074	1.037
	FIP	6.7	41.7	0.281	90.00	0.279	1.075	1.039
5	Linden	4.4	19.9	0.538	54.39	0.096	1.092	1.027
	FIP	3.8	18.8	0.475	54.00	0.067	1.104	1.021

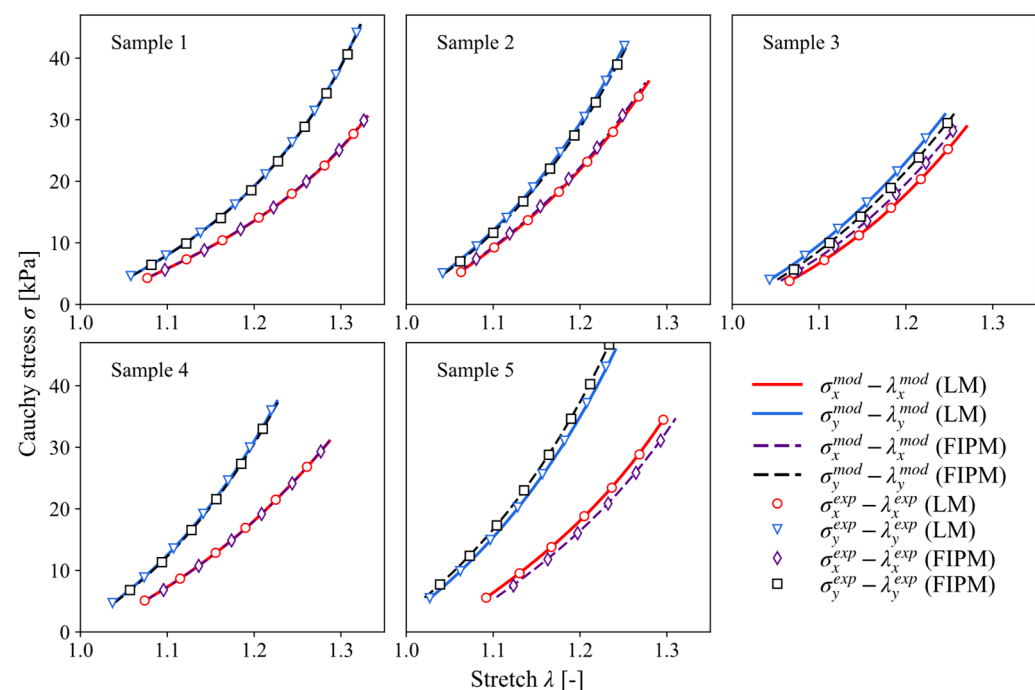


Figure 9. Comparison of stretch–stress behavior modeled with ‘Linden method’ and the fitting including prestretch method with corresponding experimentally updated data.

Good agreement between the results of both methods provides an additional validation of the newly proposed fitting method. Small deviations between results, as is the case with specimens 3 and 5, might be caused by the mutual dependency of prestretches and material parameters that are acquired through different optimization algorithms. In this study, a trust-region reflective algorithm is used, while Linden et al. [23] reported the use of the Levenberg–Marquardt algorithm.

3.3. Fitting Data from Biaxial Experiments

Data gathered from biaxial experiments on human aorta samples (described in Section 2.4 and shown in Figure 5) were analyzed using the three fitting methods. The analyses were rerun five times for each method, with different initial guesses, in the same fashion as with virtual experiments (see Section 3.1). The obtained results have proved to be independent of the initial guess choice. The results of all analyses conducted are reported in Table 6.

Table 6. Sets of material parameters obtained from fitting with three methods for biaxial experiments on human aorta samples.

Specimen	Method	C_1 [kPa]	k_1 [kPa]	k_2 [-]	α [°]	κ [-]	λ_x^p [-]	λ_y^p [-]	R^2
Donor 1	SF	14.51	4.255	127.87	90.00	0.210	-	-	<0
	SFS	11.55	0.768	39.07	61.00	0	-	-	<0
	FIP	12.21	0.37	31.35	60.88	0	1.003	1.028	0.99
Donor 2	SF	30.53	57.85	171.20	90.00	0.239	-	-	<0
	SFS	24.90	65.80	174.57	90.00	0.243	-	-	<0
	FIP	25.87	39.78	139.88	90.00	0.241	1.009	1.016	0.99
Donor 2—intima	SF	3.78	4.68	76.73	90.00	0.003	-	-	<0
	SFS	2.13	39.65	374.63	90.00	0.221	-	-	0.87
	FIP	2.14	26.88	268.30	90.00	0.206	1.017	1.004	0.99
Donor 2—media	SF	5.28	10.21	2.57	37.74	0	-	-	0.96
	SFS	2.78	13.35	2.06	39.26	0	-	-	0.98
	FIP	2.98	12.47	1.88	39.00	0	1.009	1.010	0.99
Donor 2—adventitia	SF	2.70	0.41	31.04	52.40	0	-	-	<0
	SFS	1.99	0.62	27.77	51.32	0	-	-	<0
	FIP	2.10	0.41	23.85	51.21	0	1.009	1.019	0.99

Unlike in virtual experiments, ground-truth material parameters are unknown for human aortic tissue. Opposed to the standard fitting methods, fitting with the prestretch method has been able to obtain the ground-truth material parameters from the virtual experiment data. Thus, further comparisons of fitting methods are made with respect to the solutions of fitting, including the prestretch method. Note, however, that results obtained with fitting, including the prestretch method, are not the ground-truth solutions but are, rather, considered the closest to it regarding all three fitting methods.

Material parameters calculated with the standard fitting method overestimate elastin matrix stiffness, while a subtraction of initial forces before standard fitting leads to underestimation of elastin matrix stiffness. This behavior is expected due to the preprocessing of measured forces. The stiffness of the collagen fiber family, expressed with k_1 , was greater when subtracting initial forces prior to standard fitting except for the Donor 1 specimen. The comparison of obtained sets of material parameters through the MAE is improper for experimental data since the ground-truth state cannot be determined independent of the fitting procedure.

Naturally, prestretches were calculated by utilizing the fitting with the prestretch method. The maximum calculated value was approximately 2.8%, although the mean value was much lower, being 1.24%. Material parameters obtained with the fitting with prestretch method successfully fitted experimental updated data with $R^2 > 0.99$. Sets of material parameters obtained with standard fitting methods provided a high-quality fit ($R^2 > 0.98$) to their respective measured data. However, calculating model stresses with those material parameters on experimental data updated with prestretches showed poor results. All five cases resulted in an overestimation of modeled stresses, which can be seen in Figure 10, particularly for specimens from Donor 1, Donor 2, and Donor 2—adventitia. All fitting cases converged to a solution where one of the structural parameters takes its boundary value. Although based on tissue structure, the GOH material model is a phenomenological hyperelastic model that has mutually dependent material parameters. Therefore, different sets of material parameters can provide an equally accurate fit. Furthermore, the different orientations and dispersions of fiber families in the layers of arterial tissue suggest that a single value for the entire tissue may not be informative.

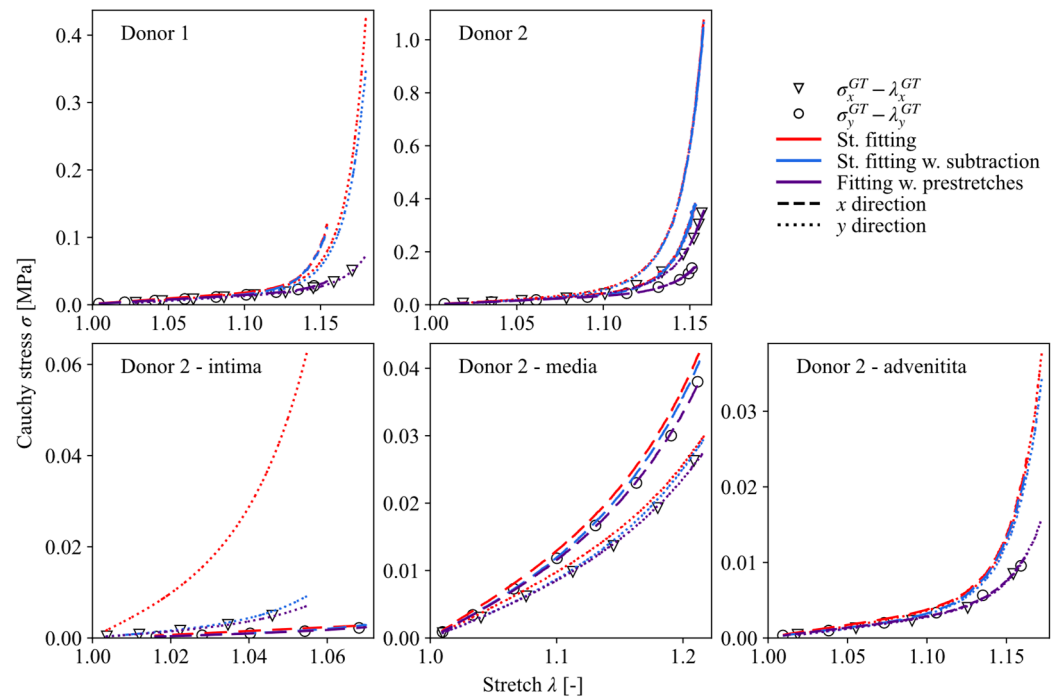


Figure 10. Stresses modeled with sets of material parameters obtained with three fitting methods on experimental data updated with prestretches for human aorta specimens.

Modeling stresses with material parameters from the standard fitting procedure and experimental stretches, updated with prestretches calculated from fitting, including the prestretch method, often resulted in a negative value of R^2 (denoted in Table 6 as <0). The calculated coefficients of determination signify a very poor correlation between modeled and experimental stresses. This occurrence is likely due to the increased value of the exponential parameter k_2 , obtained by standard fitting procedure, that is responsible for the excessive stiffening effect. Lower stretch ranges seem to be in a good correlation for most cases (excluding Donor 2—intima), but at higher stretch levels, there is a significant increase in stress values modeled with standard fitting solutions. The described behavior can also be noted in Figure 10.

4. Discussion

The goal of this study was to investigate the importance of mounting stretches in the estimation of material parameters and to establish a novel fitting method that incorporates these prestretches that occur during the mounting and positioning of the specimen prior to the execution of biaxial tensile experiments. During the experimental process, it is inevitable to slightly load the specimen before the calibration of the strain tracking system; therefore, it is not possible to measure mounting stretches. Consequently, the mounted configuration does not correspond to the unloaded reference configuration. In the newly proposed method, their connection was described by introducing the additional deformation gradient, leading to a necessity to determine an additional two parameters, i.e., mounting or prestretches. The proposed fitting method is complementary to the method proposed by Linden et al. [23], but the main difference is dividing the calculation of prestretches and material parameters into two steps, thus reducing the optimization problem complexity.

In order to verify the proposed fitting method, a series of virtual biaxial experiments were conducted using FE simulations with predefined sets of material parameters as ground-truth values. These values were chosen from the literature from experiments on human aorta. Furthermore, a comparison is made between the proposed and standard fitting methods. The findings have shown that the standard fitting method without subtraction

of initial forces yields inadequate material parameters. Subtracting initial forces prior to standard fitting significantly decreases the errors. For an ideally linear model, neglecting both mounting forces and stretches would be sufficient. However, both standard fitting methods were not able to obtain ground-truth sets of material parameters, especially for more non-linear mechanical behavior. A proportional relation between prestretches and error in obtained material parameters with standard fitting methods has been noticed. A similar observation is made for a vice versa case, i.e., for some amount of prestretch, increasing the experimental stretch reduces the error as prestretches tend to become negligible. Therefore, when conducting experiments, one should be very careful while mounting the specimen, as high initial loads can significantly influence the results.

It is also important to note that by using standard fitting methods and neglecting prestretches, one cannot obtain total experimental stretches. In contrast to standard fitting methods, the newly proposed fitting method successfully calculated prestretches as well as ground-truth material parameters. The number of iterations required to determine the solution was around 10, depending on the analyzed case, and results were obtained in realistic time, making the proposed method computationally inexpensive.

The proposed fitting method was also validated and compared to the method by Linden on experimental data available from a study [34]. Both methods were able to yield similar results with equivalent goodness of fit, despite high levels of prestretches that went even up to 10%. The fitting method proposed in this work could be more advantageous when it comes to material models with a large number of parameters since it does not directly include prestretches as the optimization variables. They are rather calculated separately from the difference between experimental and modeled stresses at timestep zero.

It is important to remember that there is a high level of uncertainty during the identification of soft tissue material parameters. The GOH material model has a large number of parameters that are not necessarily independent, and multiple equally accurate material parameter sets can be determined. However, all fitting methods were restarted multiple times and have converged to the same solutions. This supports the robustness of the selected optimization algorithm (i.e., trust-region reflective).

In proposed method, two additional parameters (λ_x^p and λ_y^p) are not optimization variables, as they are determined separately using root finding problem. On the other hand, in Linden's method, mounting stretches are computed using the non-linear least squares method. This can lead to an increased number of satisfactory solutions but also increases uncertainty.

Biaxial experiments conducted on human aorta samples have also been analyzed with the standard fitting method and the fitting that includes the prestretch method. The samples were stretched up to 20% maximum, depending on their tensile strength. The calculated prestretches were only up to approximately 3% but nevertheless had a great influence on estimated material parameters and model stresses. Note that for high stretches, stresses can be overestimated more than five times. Thus, for highly non-linear mechanical behaviors of soft tissues, the standard fitting method, even if the mounting forces are annulled, is highly inaccurate.

It was not always possible to obtain a set of material parameters without one structural parameter having its boundary value with neither standard methods nor fitting with the prestretch method. Such results are expected when testing specimens of arterial tissue as a whole since each arterial layer has fiber families oriented differently. The same observations have been made for the results of experiments on individual arterial layers [35]. This can be artificially adjusted by fixing one of the structural parameters to the value obtained from the histological measurements. Note that structural parameters are not completely independent, and unless the dispersion parameter is set to an overly isotropic value, collagen fiber angle can be calculated to ensure low fitting error. Thus, conclusions about the structure of the layers cannot be made from the optimization procedure, but rather, they should be inspected with histological microscopic analysis. Although the GOH model

is based on the structure of the tissue, it is still phenomenological and, as such, it can have multiple sets of material parameters that describe stretch–stress behavior equally accurate. This can be demonstrated by fixing an in-plane dispersion parameter κ through a range of values and conducting fitting analyses for four other material parameters.

In conclusion, neglecting the mounting stretches for highly non-linear experimental datasets can lead to significant inaccuracies in the estimated material parameter set. The inaccurate material parameters overestimate arterial wall stiffness and result in unrealistically high computed stresses. This means that inappropriate conclusions, e.g., when predicting rupture, can be drawn as a result of FE simulations made with inaccurate input. To avoid inaccuracies in the estimation of material parameters, we advise careful experimental testing and that mounting stretches must be considered during the fitting process.

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