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Characterization of regional deformation and material properties of the intact explanted vein by microCT and computational analysis

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Abstract

Purpose—Detailed mechanical information of the vein is important to better understand remodeling of the vessel in disease states, but has been difficult to obtain due to its thinness, unique geometry, and limitations of mechanical testing. This study presents a novel method for characterizing deformation of the intact explanted vein under physiological loads and determining its material properties by combining high-resolution imaging and computational analysis.

Methods—High-resolution CT (microCT) was used to image an iodine-stained, excised porcine internal jugular vein sample under extension to 100% and 120% of *in situ* length, and inflation and 2, 10, 20 mmHg of pressure, inside a microCT-compatible hydrostatic loading chamber. Regional strains were measured with the finite element (FE) image registration method known as Hyperelastic Warping. Material properties were approximated with inverse FE characterization by optimizing stiffness-related coefficients so to match simulated strains to the experimental measurements.

Results—The observed morphology and regional strain of the vein were found to be relatively heterogeneous. The regional variability in the measured strain was primarily driven by geometry. Although iodine treatment may result in tissue stiffening, which requires additional investigation, it is effective in allowing detailed detection of vein geometry.

Conclusions—The feasibility and utility of using microCT and computational analysis to characterize mechanical responses and material properties of the vein were demonstrated. The presented method is a promising alternative or addition to mechanical testing for characterizing

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7. CONFLICT OF INTEREST

Arnold David Gomez, Huashan Zou, Yan-Ting Shiu, and Edward W. Hsu declare that they have no conflicts of interest.

8. STATEMENT OF HUMAN STUDIES

No human studies were carried out by the authors for this article.

9. STATEMENT OF ANIMAL STUDIES

Animal protocols followed all institutional and national guidelines for the care and use of laboratory animals including approval by the Institutional Animal Care and Use Committees at the Veterans Affairs Salt Lake City Health Care System and the University of Utah.

veins or other similarly delicate vessels in their native anatomical configuration under a wide range of realistic or simulated environmental and loading conditions.

Keywords

veins; tomography; mechanics; imaging

1. INTRODUCTION

Disorders of the vein, either developed natively (e.g., varicose veins) or after surgery (e.g., arterial vein grafts), are frequently associated with altered mechanical loading conditions on the vessel that result in compromised wall structure, luminal blood flow, or both, with potentially detrimental consequences. [1]–[3] In turn, quantitative characterization for material properties can be helpful for monitoring disease progression. For example, compliance variations indicative of development of neointimal hyperplasia can be localized with wall strain measurements in venous grafts used for arterial bypass surgery [4]. Because vessel wall dilation is a key determinant for the maturation of arteriovenous fistula used for vascular access, stiffness or stiffening of the graft can contribute to maturation failure [3], [5], [6]. The feedback pathways between increased loading and remodeling of the venous wall have been thought to be responsible for conditions like varicose veins and venous neointimal hyperplasia. [2], [3], [5], [7], [8] Because the vein wall is composed of tissues that exhibit viscoelasticity in their constitutive behavior, a precise knowledge of the relationship between its fluidic or non-fluidic environmental loading and macroscopic morphology is essential to characterize the material properties and responses of the vessel, and help elucidate the mechanisms of the remodeling process. For these reasons, biomechanical characterization of venous tissue has been performed via biaxial testing of small samples as well as inflation and extension of whole vessels [9]–[12], among other means.

Traditionally, material properties of tissues have been obtained primarily through mechanical testing, which has been instrumental in the construction of phenomenological constitutive models of many tissues, including arteries, and to a much lesser extent veins, that are in use today [9], [10], [12]. In a typical mechanical testing experiment, whole-sample deformation and load quantification are embedded in the testing apparatus by means of hardware-based displacement sensors like magnetic or optical encoders [13]. Alternatively, to characterize regional properties, the tissue is outfitted with fiducial markers, whose displacements are then tracked via, for example, photography when the tissue is subject to different loads [14], [15]. Because these examinations can be done in the laboratory where loading and environmental conditions can be precisely controlled, mechanical testing is still considered as the standard technique for detailed characterization of the material properties of many tissues including blood vessels [16]. The main drawbacks of mechanical testing of these types of tissues are its destructive nature, the loss of a blood vessel's native anatomical configuration, and inherent limitations associated with physical examinations (e.g., number and spatial resolution of the measurements) [13].

Because of their noninvasive or nondestructive nature, imaging-based methods are increasingly used for tissue deformation and material characterization. For example, magnetic resonance imaging (MRI) and ultrasound imaging have been used to track regional tissue deformation based on the inherent tissue contrast via the so-called texture correlation and speckle tracking analyses, respectively [4], [17]. Trackable patterns in the form of grid patterns can also be generated in a process called “tagging” in MRI [18]. Alternatively, regional deformation of tissues is directly measured via motion-sensitive imaging such as phase-contrast velocity and displacement-encoding MRI, and Doppler ultrasonography [19], [20]. Although computed tomography (CT) provides the highly desirable combination of spatial resolution, image quality and acquisition speed, it has been seldom utilized due to its limited soft-tissue contrast. However, in light of recent advances in specimen staining techniques that make otherwise undetectable details of tissues visible [21], [22], CT-based deformation measurements are now conceptually feasible and worth exploring. The 3D feature offered by CT is especially desirable as basis for comprehensive mechanical analysis, since the morphology and, thus, mechanical responses of veins occur in all spatial directions.

Besides image acquisition, an integral part of imaging-based deformation characterization is the underlying computational analysis. Image processing is often used to enhance and isolate features of interest necessary for the identification of tissues and markers as deformation takes place [23], [24]. Further, image registration methods, utilizing rigid or non-rigid deformation, have been employed to replace visual inspection for automated tissue tracking, which can be obtained with image correlation [17], or similarity measures like mutual information [25] and local contrast [26], [27]. Although each deformation type and registration metric can be advantageous in different specific applications, in general, the accuracy of image registration in capturing the underlying motion increases as more degrees of freedom are allowed in the deformation. To counter numerical instability associated with ill-conditioning due to increasing degrees of freedom, the registration formulation is often regularized with physics-based processes, such as diffusion, fluidic advection, or solid deformation [26]–[28].

The advent of computational image analysis has also made it possible to approximate the constitutive behavior of tissues by means of inverse finite element (FE) approaches, which can provide a way to circumvent the physical limitations of mechanical testing. Whereas traditional forward FE simulations are used to predict tissue deformation according to conservation principles based on given information about load conditions and constitutive behavior, inverse parameter estimation seeks to infer material parameters based on known loads and deformation [29], [30]. Image-based inverse material characterization, counter-intuitively, is performed by iterative material property optimization using forward simulations, and has been applied in soft tissues, including the liver [31] and the heart [32]. Provided that images of sufficient resolution and contrast are obtainable for accurate quantification of the local tissue strain [29], and the range of strains resembles biaxial data, i.e. like in extension-inflation vessel analysis, this technique is expected to be uniquely beneficial for material characterization of vein samples, especially in diseased vessels where even small changes can be significant but difficult to detect via physical examinations.

The overall purpose of the study is to investigate the feasibility of novel means of high-resolution imaging with computational analysis to perform functional and material characterization in thin-walled veins. This includes the development of experimental protocols for using high-resolution CT, or microCT, to capture local microscopic-scale features of vein specimens in 3D under load, and application of computational analysis on images obtained under a controlled-loading scenario to quantify deformation and material properties of the vessel. The experimental considerations include the description of a microCT-compatible experimental setup and tissue contrast enhancement procedures. On the computational side, this study demonstrates the use of image registration for strain measurement in these types of images, and subsequent material characterization via inverse FE analysis. Hyperelastic Warping [28], [33], [34] was selected as the means for image registration because it is integrated with FE analysis, making it particularly convenient for the current study where the material modeling parameters are themselves the subject of investigation.

2. METHODS

2.1 Imaging

2.1.1 MicroCT-Compatible Pressurization Apparatus—Based on previous evaluations of large artery mechanics under pressure and other loads [14], [35], [36], a microCT-compatible apparatus was constructed to provide varying physiological pressurization of the vein. The system has small physical size to allow close rotation of the x-ray source required for high-resolution CT acquisition, and is mostly made out of plastic material to reduce artifacts associated with large attenuation coefficient differences [37]. Figure 1 shows a schematic of the entire apparatus designed to accommodate veins of various diameters. The sample was mounted on miniature barb adapters and secured with surgical thread at each end of the phosphate-buffered saline (PBS)-filled test chamber. The adapter was designed to allow extension of the vein sample as well as pressurization, and was fitted with a removable plug for purging air bubbles. Hydrostatic pressurization ranging from 0 to 180 mmHg was achieved via a PBS column, whose level was maintained by the small flow (0.5 ml/min maximum) introduced by a roller pump to compensate for vessel permeability or inadvertent leaks at the tissue-adapter connections.

2.1.2 Sample Preparation—Animal protocols were approved by the Institutional Animal Care and Use Committees at the Veterans Affairs Salt Lake City Health Care System and the University of Utah. One external jugular and one subcutaneous abdominal vein samples, each about 30 mm in length, were harvested from 3-month-old female Yorkshire cross domestic pigs (Sigma Livestock, USA) used as control animals at the end-time points of an unrelated study. Due to logistical constraints, the subcutaneous abdominal vein was used immediately after excision, whereas the jugular specimen was frozen (-80°C) and used within 3 h from thawing before changes mechanical properties take place [38].

Although the relationship between pretreatment with Lugol's solution and soft tissue contrast has been established in previous micro-CT visualization of embryonic and cardiovascular tissues [21], [22], the protocol needs to be optimized for vein specimens. To

this end, the subcutaneous abdominal vein sample was divided into eleven 3-mm-long sections, and one section was soaked in each 0, 5, 10 or 20% Lugol's solution (cat# 26385-06, Electron Microscopy Sciences, USA) for 1.0, 2.0, or 3.0 h. The start times of the different soaking procedures were timed such that the sections could be imaged together. For the stretching and pressurization experiment, the jugular vein sample was pretreated using the duration and concentration deemed minimally sufficient to produce detectable contrast (5% for 1 h).

2.1.3 MicroCT—For the pretreatment optimization experiment, the vein sections were placed inside a PBS-filled 3.0 ml syringe serving as sample holder, and imaged on a Siemens Inveon instrument (Siemens Medical Solutions, PA, USA). Three-dimensional images of $23 \times 23 \times 55$ mm field-of-view with $17 \mu\text{m}$ voxels were acquired using 701 projections with the source operating at 80 kVp and $120 \mu\text{A}$, which took 60 min. In post-processing, the image contrast, for each vein section was quantified by,

$$\text{contrast} = \frac{H_t - H_b}{H_b}. \quad (1)$$

where H_t and H_b were the average Hounsfield intensities of the tissue and the background, respectively.

For the pressurization experiment, the pretreated sample was rinsed in PBS, mounted on the pressurization apparatus, and imaged using the same protocol, except with reduced resolution ($100 \mu\text{m}$) to minimize scan time (18 min) to minimize possible contrast reduction due to diffusion. Jugular vein was first imaged in its unloaded state, which corresponded to approximately 60% shrinkage from its *in situ* length. The sample was then stretched to its estimated *in situ* length (100%) and imaged at each 2, 10, and 20 mmHg pressurization. Finally, the vein was stretched to 1.2 times its *in situ* length (120%), and imaged at the same pressure levels.

2.2 Computational Mechanics

For completeness, some useful theoretical concepts are briefly reviewed. In equations, vectors are identified with arrow accents and tensors with underbars.

2.2.1 Theoretical Background—In this study, deformation was quantified by comparing tissue coordinates in a reference (i.e., unloaded) configuration, $\vec{X}$, with corresponding locations in a current (deformed) configuration, $\vec{x}$, via the Right Cauchy strain tensor[39],

$$\underline{C} = \left(\frac{d\vec{x}}{d\vec{X}} \right)^T \frac{d\vec{x}}{d\vec{X}}, \quad (2)$$

which is used to define constitutive modeling relationships. For visualization, the circumferential component of the Green-Lagrange strain tensor,

$$\underline{E} = \frac{1}{2} [\underline{C} - \underline{1}] \quad (3)$$

($\underline{1}$ indicates the identity matrix), was shown in color maps.

In hyperelastic materials, often used to model venous tissue [9], [10], a scalar strain energy function, W , may be used to relate strain to stress, via the second Piola-Kirchoff stress tensor[39],

$$\underline{P}=2\frac{\partial W}{\partial \underline{C}}, \quad (4)$$

which allows the formulation of constitutive material models by defining a strain energy function, W , that fits experimentally obtained strain-stress responses, or predictions of deformation of an FE model under idealized loads.

Strain measurement via image registration is commonly achieved by finding the deformation map that minimizes a given measure of similarity between two images [26]–[28]. Assuming intensities represent body points in reference and current configurations, images acquired before and after a deformation can be expressed as scalar fields known as the template,

$T(\vec{X})$, and target $S(\vec{x})$, respectively. By comparing local intensity differences, it is possible to construct a scalar similarity measure that approaches zero as one image is deformed into the other. One such metric is the image energy,[26], [28]

$$U(\vec{X}, \vec{x}) = \frac{1}{2} [T(\vec{X}) - S(\vec{x})]^2. \quad (5)$$

Compared to other registration strategies using Equation 5, Hyperelastic Warping is unique in that the desired deformation field minimizes the image energy, U , along with the material-specific strain energy function, W , by means of the FE method. [28], [33], [34] Thus, given a set of images, geometrical, and constitutive FE modeling, the main criterion for finding $\vec{x}$ (or strain via Equations 2-4) is the minimization of the net energy ψ of a system in volume β ,

$$\Psi(\vec{x}) = \lambda \int_{\beta} U(\vec{X}, \vec{x}) dv - \int_{\beta} W(\vec{X}, \vec{x}) dv, \quad (6)$$

where λ weights the relative contribution of image information. As formulated, image energy terms effectively provide loading by means of body forces, which are consequently equilibrated by internal stresses associated to model deformation according to its constitutive and geometrical characteristics. [33], [40]

2.2.2 Geometrical and Constitutive Modeling—To construct the FE model required for deformation measurements, the sample's geometry was obtained from images acquired at the unloaded configuration by segmentation (Amira, FEI Visualization, Germany), and rendered in hexahedral-elements (True Grid, XYZ Scientific Applications, USA). To minimize segmentation error resulting in geometrical noise, boundary surfaces were smoothed before rendering (MeshLab, ISTI-CNR, Italy). The model consisted of 9250 elements—37, 125, and 2 along the vessel circumference, length, and wall thickness, respectively. Local mesh refinement was applied to preserve element quality and sample

geometry. Mesh density was tested with a maximum-strain convergence analysis by simulating 60-mmHg pressurization at 100% *in situ* length with 3, 6, 9, 15, and 23 thousand-element meshes.

The jugular vein tissue was modeled as anisotropic fiber-reinforced composite based to the microstructural model described in [41] where tissue response was represented by exponential fibers embedded in a nonlinear matrix. For its finite element analysis, the matrix was implemented using the nonlinear description by Veronda & Westmann [42] with the strain energy,

$$W_{matrix} = c_1 \left[e^{c_2 (\bar{I}_1 - 3)} - 1 \right] - \frac{c_1 c_2}{2} \left[\bar{I}_2 - 3 \right] + \frac{K}{2} (\ln J)^2, \quad (7)$$

with $J = \det \left(d\vec{x} / d\vec{X} \right)$, $\bar{I}_1$ and $\bar{I}_2$ as first and second invariants of the deviatoric Right Cauchy strain tensor ($\underline{C}_{dev} = J^{2/3} \underline{C}$ from Equation 2) [39], and the following material-specific coefficients: c_1 (in kPa) and c_2 (dimensionless), which regulate stiffness, and K (in kPa) as bulk modulus. The four fiber families were implemented as unidirectional exponential reinforcements with strain energy density given by,

$$W_{fibers} = \sum_{m=1}^4 \frac{\xi^{(i)}}{2\alpha^{(i)}} \left(\exp \left[\alpha^{(i)} \left(\bar{I}_n - 1 \right)^2 \right] - 1 \right), \quad (8)$$

where $\xi^{(i)}$ and $\alpha^{(i)}$ correspond to material coefficients of the i -th fiber $i \in \{1, 2, 3, 4\}$ and $\bar{I}_n$ is the projection of the Right Cauchy strain tensor along the fiber direction (the square of the fiber stretch). Fiber directionality was controlled by the angular distance, $\phi^{(i)}$, within the local element coordinate system with $\phi^{(3)} = 0^\circ$ corresponding to circumferential, $\phi^{(3)} = 90^\circ$ to longitudinal, and $\phi^{(1)} = -\phi^{(2)}$ to oblique fibers (it is also worth noting that $\xi^{(1)} = \xi^{(2)}$ and $\alpha^{(2)}$), respectively. The cumulative response of the fibers and the matrix was implemented as an uncoupled mixture, where the strain energy density of the composite material was $W = W_{matrix} + W_{fibers}$ [43], [44], given the assumption of near-incompressible behavior enforced by a relatively large bulk modulus of 5000 kPa. To evaluate the influence of material assumptions on the strain measurement, three sets of material coefficients were obtained by least-squares fit of anisotropic stress-strain curves (Equations 7 and 8) to published experimental data from seven jugular vein samples (Sokolis et al. 2012, Figure 2) [10]: the first set corresponds to fitting the model response to the mean strain-stress relationship and is henceforth referred as “*baseline*” material. The second and third sets correspond to best fits of the model response to the upper and lower experimental ranges producing “*compliant*” and “*stiff*” materials, respectively. Response curves for the three material sets are shown in Figure 2. (Material coefficients are presented in Table 1).

2.2.3 Strain Measurement via Hyperelastic Warping Analysis—Strain measurements at each of the loading scenarios were performed with each of the material responses (“baseline”, “compliant”, and “stiff” materials, respectively) to determine if image-based strain measurements via Hyperelastic Warping were independent of material properties and could be used for inverse parameter identification. To this end, Hyperelastic

Warping was performed as implemented and described previously [40], [45], [46] using the FEBio software suite [43]. The reference configuration was defined as the unloaded configuration and its corresponding microCT imagery was used as the template volume for Hyperelastic Warping. The images from each of the loading scenarios were used as warping targets. For large deformations, small auxiliary loads were implemented to place the model in the vicinity of the deformed configuration and then increasing the image weighting parameter, λ , (Equation 6) until registration forces are predominant and drive the system towards registration. Differences in deformation results were identified by strain map comparison, and with the use of scatter plots.

2.2.4 Material Property Estimation—Ideally, material properties could be inferred by iteratively running physics-based simulations driven by loads equivalent to those applied experimentally, comparing the resulting strain field to that observed empirically, and using the difference as feedback. For example, a simulated strain field that was lower than experimental measurement would indicate that the stiffness in the model was too high, and vice versa [29], [30]. In practice, the existence and uniqueness of an optimization solution could be complicated by the large number of optimization variables [47]. For simplification, parameter identification was reduced to scaling coefficients for the matrix parameter c_1 , and for modification of the fiber coefficients $\xi^{(i)}$, and $\alpha^{(i)}$ in order to influence the low and high range of load-dependent stiffness, respectively.

Physics-based FE simulations for optimization utilized the geometry described in the Computational Modeling section, but were not driven by image information. Instead, the forward analyses were used to simulate each of the loading scenarios, under restricted nodal displacements, or fixed boundary conditions, at the ends of the sample similar to the pressurization apparatus (labeled *BC* in Figure 5, C and E). Axial deformation was prescribed in the code, but longitudinal force data could be used when available. The difference between the Green-Lagrange strain tensor components found experimentally via Hyperelastic Warping and forward simulation, E_{ij} and $\hat{E}_{ij}$, respectively, was quantified by the total residual RMS, $\varepsilon = \sum \varepsilon_m$. In turn, the residual RMS for the m -th loading scenario investigated, $m \in \{1, 2, 3, 4\}$, representing 100% and 120% extension under 10 and 20 mmHg pressurization over N elements in the analysis region was calculated by,

$$\varepsilon_m = \sqrt{\frac{1}{N} \sum_{n=1}^N \left[\sum_{i=1}^3 \sum_{j=1}^3 \left(E_{ij}^{(n)} - \hat{E}_{ij}^{(n)} \right)^2 \right]}. \quad (9)$$

To avoid the influence of edge effects, strains at the end of the sample were excluded, and the residual RMS computation was performed in the middle region (Figure 5F). Minimization of the total residual RMS, ε , using a multivariable derivative-free method [47] was scripted in Matlab (The Mathworks, USA) and interfaced with FEBio, which was used for forward simulations. For interpretation of results, the load-specific residual RMS, ε_m , was used as a measurement of the quality of the forward simulations for each loading scenario.

3. RESULTS

Figure 3 shows microCT slices of the subcutaneous abdominal vein sections pretreated with different concentrations of Lugol's solution for different durations, along with local contrast quantifications. As expected, contrast increased with either concentration or duration, albeit each relationship is not proportional. Maximum contrast, 0.83, was obtained by soaking specimen sections for 2 h in 20% Lugol's solution. The contrast achieved in all the pretreated specimens was deemed sufficient for image segmentation. Although not all of the treatments were used for the pressurization experiments, structural features desirable for image registration (e.g., texture or brighter areas) can be seen in all samples, especially in the tissue treated with relatively higher concentrations ($>10\%$). To reduce possible tissue shrinkage or material property alterations associated with higher concentrations [21], the treatment with lowest concentration and shortest soaking time (5%, 1h) was selected for use in the pressurization experiments.

Selected microCT slices of the jugular vein sample at the unload configuration and the investigated loading scenarios are shown in Figure 4. Stretching with near-zero pressure loading produced visible reduction in the vessel diameter and wall thickness, as expected due to tissue incompressibility. Neither vessel collapse was observed at low pressurization, nor was buckling seen during hypertension. The sample spanned approximately 900, 100 μm -thick slices, but because its ends were tied to the adapters, only center slices (about 640) showed conspicuous deformation characterized by larger luminal diameter, wall thinning, and rounder circumference compared to the unpressurized states. Figure 4 also contains some additional features, such as a remnant of connective tissue, a small air bubble, and imaging artifacts (as noted in the Figure caption), but these were not included in the generated model geometry and hence had minimum impact on the results.

In the construction of the computational model, mesh density convergence was determined to occur with 9,250 elements, because a 5% change in strain maxima was observed compared to the solution using 15,000 elements. For computational efficiency, given the large number of simulations needed for the study, the 9,250-element-mesh was used for subsequent analysis. Average computational time for forward simulations and Hyperelastic warping was approximately 45 min on a 12-core, 2 GHz machine (AMD Opteron 6234).

Figure 5 shows longitudinal microCT slices, 3D mesh renderings, and photos (obtained after the pressurization experiments) of the internal jugular vein specimen in two of the deformed configurations, 100% to 120% extension at near-zero pressurization, which are representative of the other loading scenarios (not shown). The computational model captured the macroscopic deformation of the specimen, as evident in the microCT and optical imagery. Moreover, some large microscopic morphological features unique to the sample under investigations, like variations in diameter, also deformed consistently with visual inspection of the images. Microscopic morphological features are expected to contribute to regional strain distribution.

Representative results of the Hyperelastic Warping analysis are shown in Figure 6, which includes circumferential strain maps and comparative scatter plot of the x, y and z

components of the Green-Lagrange strain obtained for 100% extension with 20 mmHg pressurization. Despite using three distinctly different material coefficient sets, the circumferential strain maps are highly similar with few noticeable regional differences (e.g., at noted locations on the strain maps). Overall, the circumferential strain varied from approximately 0.11 to 0.60 for the vessel exterior, and 0.29 to 0.59 for its interior. To a first approximation, the ranges of strain observed on the vessel wall are consistent with the increase of the vessel outer diameter (from approximately 3.2 mm in the unloaded state to 3.8 mm in the stretched and inflated state) seen in the CT cross-sectional images (see Fig. 4). Generally, similarly consistent patterns were also observed in the responses at different loading scenarios (not shown).

Figure 6 also shows scatter plots directly comparing selected Green-Lagrange strain components (E_{xx} , E_{yy} and E_{zz}) between different material assumptions (“compliant” versus “baseline”, and “stiff” versus “baseline”). The high coefficients of determination, $R^2 = 0.98$, of the scatter plots are consistent with the highly similar strain maps. The independence of strain results from material assumptions is a direct consequence of imaging information playing the predominant role in Hyperelastic Warping, which does not preclude minor differences due to the need for fine tuning the registration parameter for each material. Not surprisingly, the general trend in the scatter plots indicates that softer materials slightly overestimated strain, consistent with observations in the circumferential strain maps. Scatter plots of other strain tensor components (not shown) followed the same trends. These small differences translated to a residual RMS of approximately 0.05 across all components of strain tensor from the investigated loading scenarios, in agreement with a prior study of tissue inflation using the same technique and similar image quality [45].

Figure 7 shows circumferential strain maps from physics-based FE simulations using different material coefficient sets, and comparison of strain results of simulated strain results with respect to experimental measurements. The results of “baseline” coefficients can be readily inferred from the behaviors the “compliant” and “stiff” models shown (Figure 7A and 7B). In general, under identical loads, both the “compliant” and “stiff” models result in markedly more deformation. The trend of behavior is consistent with the scatter plots, where the similarity between strains obtained by forward simulations and Hyperelastic Warping increases with the relative stiffness of the material being tested.

As expected, the circumferential strain map obtained using the optimized material parameters (Figure 7C) shows good agreement with the empirical measurements (Figure 6A, 6B, and 6C) over a majority, roughly 70% of the surfaces displayed, with isolated areas of larger disagreement. The optimization procedure reduced the cumulative residual RMS from an initial value of 2.52 in the “baseline” material to a final value of 1.02, which affords a quality improvement in simulations from 0.63 to 0.19 in the 100%-stretch 10 mmHg pressurization, 0.90 to 0.43 in the 100%-stretch 20 mmHg, and 0.73 to 0.21 in the 120% 20 mmHg pressurization, but slightly elevated simulation residuals for the 120%-stretch 10 mmHg pressurization scenario (RMS increase from 0.41 to 0.46 per Eq. 9).

The optimized parameters are $c_1 = 5.77$, $\zeta^{(1,2)} = 3.11 \times (10)^{-6}$, $\zeta^{(3)} = 6.93 \times (10)^{-7}$, $\zeta^{(4)} = 1.01 \times (10)^{-5}$, and $\alpha^{(1,2)} = 5.22$, $\alpha^{(3)} = 4.47$, $\alpha^{(4)} = 0.73$, which roughly indicate an increase of matrix relative stiffness (via c_1) by 300% and fiber stiffness of 30%.

4. DISCUSSION

The results above demonstrate that, an accordingly optimized protocol (Figure 3), recently developed iodine tissue-staining techniques [21], [22] can also be applied on the explanted vein. The staining pretreatment allowed the vessel's morphology (Figure 5) and function, like deformation (Figures 4 and 6), to be evaluated in 3D and high-resolution using microCT and the appropriate testing apparatus. While a voxel dimension of 100 μm is used in the present study, higher resolution is possible with specialized instrumentation. Even with the current image resolution and quality, the results reveal detailed three-dimensional geometry including vessel diameter and wall thickness non-uniformities despite the microscopic size of these features.

The present study also demonstrates the feasibility of coupling high-resolution CT-based geometrical characterization with computational analysis to examine functional behaviors of the vein sample. The image-driven strain analysis, as shown in Figure 6 shows a direct assessment of vein deformation under a loading condition that mimics its native environment and anatomical configuration. Although optical-based methods [48], [49] are also noninvasive and non-destructive, due to the limitation of light penetration, they capture only surface motion and necessitate invoking incompressibility assumptions for reconstruction of 3D information. These approaches surpass the quasi-temporal resolution of CT (i.e., intermediate imagery as pressurization occurs) making them suitable for material model form investigation. In contrast, CT imagery delivers high spatial resolution in full 3D, which allows determination of strain in complex shapes like bifurcations and fistula interfaces, which are not readily suited for optical methods. In addition to the spatial resolution in which the measurements are made, the findings suggest that image-based functional and material analysis can be a desirable alternative or adjunct to specialize a given material model to a specific specimen, particularly in situations like examination of very small and highly fragile samples without sectioning or clamping them.

Importantly, once functional characterization is complete, computational analysis based on the same geometrical and material model can be used to examine tissue responses under a variety of loading settings, including ones that are beyond physiological conditions. As shown in Figure 7, material assumptions can affect the accuracy of deformation simulations. In the present study, in order to explain the discrepancy between simulated and empirical strains, the relative stiffness of the material had to be increased. The precise cause of the apparent tissue stiffening is currently unclear, but can include material property changes caused by the iodine staining, or intrinsic differences between the tissues used to obtain the initial material model and the tissue used in this investigation. Despite of this result, qualitative agreement between simulated and measured strain distribution indicates that over the majority of the structure under investigation, the strain distribution is primarily driven by geometrical spatial variations due to vessel morphology. Additional minor differences may be attributed to spatial variations in material properties or other unaccounted modeling

assumptions. Combined, the characterization of the regional (Figures 6C and 7C) as well as macroscopic response (Figure 5), indicate that the proposed methodology is a potentially useful means for multi-scale mechanical analysis of the vein.

Being a proof-of-concept study, there are several ways in which the current work can be improved. One obvious concern relates the use of a single specimen in the current study. Although justified for the purpose of demonstration, a rigorous validation study based on multiple specimens and direct comparison to mechanical testing is needed to establish the accuracy of the measurements. Similarly, despite soaking the tissue in the lowest concentration of Lugol's solution and for the shortest duration deemed practical for imaging, whether there has been any adverse effect, and if so, whether the effect can be avoided (e.g., by further lowering exposure to the treatment solution or using an alternative solution) remain to be investigated. Another source of concern is the simplistic nature of assumptions made in the geometric and material modeling (e.g., homogeneous material properties) in order to focus on the complete analysis workflow and its feasibility. While there is general agreement between the Hyperelastic Warping results (Figure 6) and the physics-driven simulations (Figure 7) with optimized material parameters, the assumptions likely have contributed to the observed localized discrepancies. Likewise, because the reference configuration used in this study (the unpressurized state) is assumed to be completely unloaded, the forward simulations would have the tendency to overestimate deformation if pre-strain is present in the tissue. The accuracy of the predictions can likely be improved by using more sophisticated modeling.

Despite the potential limitations, findings of the current study are promising for future work in image-based computational mechanics in the vein. As a start, as shown in arteries [35], [50] and some venous tissues [10], [36], it is possible to perform a more generalized analysis including spatially heterogeneous material characterization. However, depending on its application, the number of degrees of freedom involving spatial characterization would render results susceptible to artifacts due to local minima, numerical ill conditioning, and a large computational expense. These challenges could be dressed, for instance, by parameterizing coefficients along the spatial directions, for instance using polynomial and sinusoidal functions. Further, fluidic loads may be included by coupling the presented analysis with computational fluid dynamics [5], which can be valuable for understanding the mechanisms involved in varicose vein formation and venous neointimal hyperplasia, or improving arteriovenous fistulas, hemodialysis grafts, and vessels for cardiac bypass. Finally, although demonstrated for micro-CT, the computational analysis presented here is compatible with most imaging modalities, including *in vivo* applications, provided that sufficient resolution and contrast are achievable to capture relevant morphological features in 3D. For example, Hyperelastic Warping has already been used for *in vivo* strain characterization of tissues, including ligaments [51], coronary artery [34], heart [51]. Given the continuing advances in MRI resolution, ultrasound imaging quality, and CT contrast, if not already feasible, it is just a matter of time until *in vivo* characterization of functional and material properties of the vein using this approach becomes a reality.

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Figure 1. MicroCT-compatible pressurization apparatus. The schematic (A) illustrates the overall system designed to allow imaging of the vessel under different loading conditions. The photograph (B) shows the actual study specimen mounted in the test chamber obtained after microCT imaging, with surrounding fluid and chamber cover removed for clarity. Notice the coloration of the specimen due to iodine staining.

Figure 2.

Stress-strain responses of the material models used in all computational analyses.

Anisotropic stress-strain curves (solid lines) for various degrees of extension (indicated by the specified color) reflect least squares fitted results based on empirical data from inflation-extension tests of jugular vein samples [10]. Both circumferential (a) and longitudinal (b) responses are shown. From the “baseline” responses, two material coefficient sets, “compliant” and “stiff” (dotted and dashed lines, respectively), were obtained.

Figure 3.

Dependence of CT contrast on soaking time and solution concentration. Each panel shows a vein cross section, and quantified contrast, obtained after pretreatment with different Lugol's solution concentration (vertical) and soaking duration (horizontal). Unstained specimens (top row) were invisible.

Figure 4.

Micro-CT images of a vein sample under load. The cross-sectional slices were obtained at approximately the same location of the same specimen under the specified stretching and pressurization conditions. The axial stretch is given in terms of the specimen's in vivo length, with 40% being the unloaded length. The intensity and contrast of the images have been enhanced to better visualize the vein. The bright region at the bottom of each image (1) is from a piece of remnant connective tissue attached to the vein. A small air bubble (2) and ring-like instrumentation off-center rotation artifacts (3) are also visible.

Figure 5.

Computational model geometry. The left and right columns correspond to 100%- and 120%-stretched states of the same specimen. The microCT images (A and B) were segmented to generate the vessel wall. The computational mesh representations, in whole (C and D), and cut (E and F), illustrate the relatively heterogeneous diameter and wall thickness of the vessel. In order to reduce possible artifacts arising from boundary condition locations (BC), the computational analysis performed over only the indicated region. Photos of the vessel (G and H) taken after the imaging experiment are included for reference.

Figure 6.

Hyperelastic Warping strain measurement. The whole and cut-away color-coded maps (A—C) show largely similar circumferential strain despite the 3 different sets of material assumptions used. Examples of where minor differences exist are pointed out by the matching arrows. The scatter plots (D—F) directly comparing strain tensor components obtained under different material assumptions reveal slightly overestimated and underestimated deformation when the “compliant” and “stiff” materials were used, respectively.

Figure 7.

Forward FE simulations of the pressurized vein. Whole and cut-open circumferential strain maps obtained for the vein specimen at 100% stretch and 20 mmHg pressurization using “compliant” (A), “stiff” (B), and optimized (C) coefficient sets demonstrate the dependency of simulation results on material assumptions. The scatter plots of the strain tensor components (D–F) comparing between material assumptions indicate that both “compliant” and “stiff” materials overestimate the deformation. For clarity, the number of data points in the scatter plots have been down-sampled by a factor of 10.

Table 1

Material modeling coefficients sets.

	c_1 [kPa]	c_2 [-]	$\xi^{(1,2)}$ [kPa]	$\alpha^{(1,2)}$ [-]	$\phi^{(1,2)}$ [deg]	$\xi^{(3)}$ [kPa]	$\alpha^{(3)}$ [-]	$\xi^{(4)}$ [kPa]	$\alpha^{(4)}$ [-]
"baseline"	1.94	0.60	2.65e-6	4.42	62	5.87e-6	3.78	8.22e-5	0.614
"stiff"	2.52	0.61	2.90e-6	4.86	62	6.46e-6	4.16	9.04e-5	0.67
"compliant"	1.36	0.63	1.86e-6	3.27	62	5.28e-6	3.40	7.38e-5	0.55