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Constitutive Modelling and Histology of Vena Saphena

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Abstract. The inflation-extension test was performed in order to obtain the mechanical response (stress-strain curves) of the human vein - *vena saphena magna* (usually used for coronary artery bypass graft surgery). Tubular samples of the vein were inflated four times up to the pressure approx. 4 kPa (vein pressure) and then four times up to approx. 16 kPa (systolic pressure). The experiments were recorded by the CCD camera. The longitudinal and circumferential deformations of the tube were evaluated using the edge detection method. The experimental data were fitted by anisotropic, nonlinear, constitutive model in order to obtain model parameters, especially the parameter which can be explained as collagen fibres orientation approximation. This parameter was then compared with the findings from histology. The histology analyses based on label-free imaging were performed additionally to the mechanical testing. Collagen (most important load-bearing component of the vein wall) was visualized using second harmonic generation imaging (SHG, excitation at 860 nm by a tunable IR pulse laser, detection at 430±10 nm). This method enabled us to observe collagen through the vein wall. It was found that the collagen fibres are helically aligned within the vein at an angle 37±6° measured from circumferential axis. The results of collagen orientation angle show a good agreement of findings obtained from histology and from constitutive model.

Introduction

The progress in the field of tissue engineering and clinical surgery demands further investigation of the correlation between microscopic structure and macroscopic mechanical response of the material [1]. Saphenous veins are often used as a replacement in coronary-artery and peripheral-artery-bypass graft surgery. Large attention has been paid to flow conditions and wall sheer stress which are suspected to be responsible for remodelling process and development of intimal hyperplasia [2]. Varicose veins were also studied from the mechanical and histological point of view [3,4].

However there are only few papers dealing with constitutive modelling and histology of saphenous veins used for bypass graft surgery (see e.g. [5]). The aim of this preliminary study is to extend the available set of material parameters and correlate these parameters with findings obtained by histology based on label-free method (see e.g. [6]) – second harmonic generation (SHG) imaging microscopy.

Materials and Methods

The sample of healthy human *vena saphena magna* (the longest vein in the body running along the length of the leg) was harvested from a cadaveric donor (a 50-year-old man, 26 hours post mortem) at the Department of Forensic Medicine of the Na Kralovskych Vinohradech University Hospital in Prague. The use of post-mortem human tissue was approved by the Ethics Committee of the University Hospital. The vein was immediately stored in the physiological solution and tested within four hours after excision. The second section of the vein was visualized for type I collagen in order to analyse the dominant direction of this load-bearing protein within the vein wall.

Inflation-extension test. The sample was mounted into the inflation test setup (Fig. 1) and underwent quasistatic inflation by internal pressure. The specimen of *vena saphena* was inflated four times up to the pressure approx. 4 kPa (vein pressure) and then four times up to approx. 16 kPa (systolic pressure).

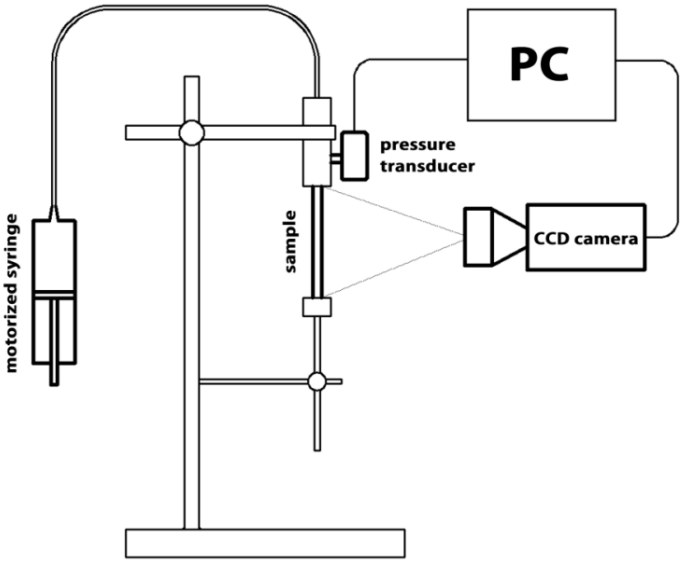

Fig. 1 Inflation-extension test set-up.

The intraluminal pressure was recorded during the test by the pressure transducer (Cressto, Cressto s.r.o. Czech Republic). The marks were created on the surface of the vein in order to evaluate longitudinal deformation. The sample was recorded by CCD (charge-coupled device) camera (Dantec Dynamics, Skovlunde, Denmark) and the diameter and axial distance of the marks in loaded configuration was evaluated using the edge detection method. The longitudinal (λ_z) and circumferential (λ_t) stretches of the tube were computed via Eq. 1 and Eq. 2 assuming the incompressibility of the venous wall expressed by Eq. 3.

$$\lambda_t = \frac{2\pi r_m}{2\pi R_m} \tag{1}$$

$$\lambda_t = \frac{l}{L} \tag{2}$$

$$\lambda_r = \frac{1}{\lambda_t \lambda_z} \tag{3}$$

Here r_m and R_m are middle radii of the tube in deformed, respectively reference geometry. l and L is longitudinal distance of marks in deformed and reference configuration.

The experimental circumferential (σ_{tt}) and longitudinal (σ_{zz}) stress during the loading was computed adopting presumption of the thin-wall geometry with closed ends which results in Laplace's Law Eq. 4, 5.

$$\sigma_{tt}^{EXP} = P\frac{r_m}{h} = P\frac{R_m\lambda_t^2\lambda_z}{H} \tag{4}$$

$$\sigma_{zz}^{EXP} = \frac{\sigma_{tt}}{2} \tag{5}$$

Here P is intraluminal pressure; H is the reference thickness of the sample. The reference geometry of the sample is listed in Table 1.

Table 1 Reference geometry of the sample

Outer radius	Thickness	Distance of axial marks
Ro [mm]	*H [mm]*	*L [mm]*
2.21	0.42	9.50

Histological Analysis. The backward non-descanned second harmonic generation imaging was used to visualize type I collagen (fibrillar protein) (see e.g. [7]). The sample was excited by IR pulse laser at 860 nm wavelength and the emitted signal detected at 430±10 nm (microscope LEICA SP2 AOBS, Leica Microsystems, Wetzlar, Germany). SHG in contrast to classical histology enables one to observe type I collagen through the vein wall in native configuration without any staining and preparation (fixing, cutting) of histological slices. The digital image of type I collagen formed by SHG imaging microscopy and the definition of the fibre angle β are shown in Fig. 2.

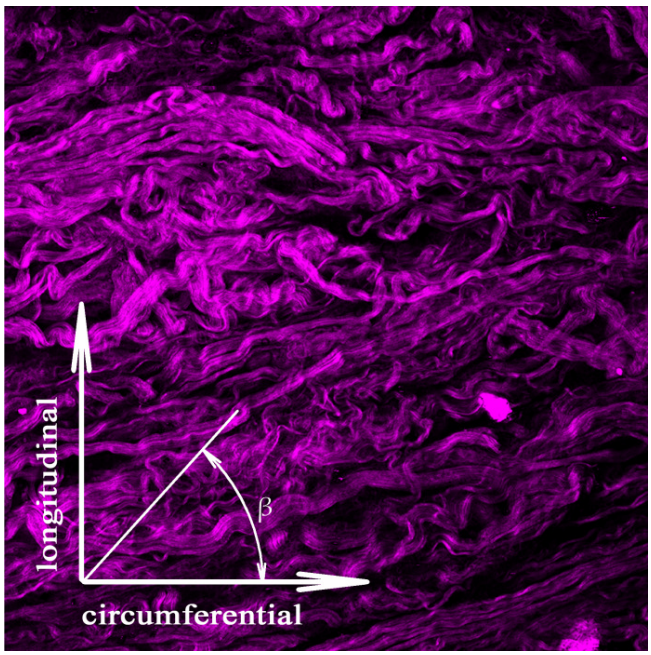

Fig. 2 *Vena saphena magna* visualized for type I collagen and the definition of fibre angle β.

Histological images were evaluated using BinaryDirections software, developed in-house, which implements the Rotating Line Segment (RoLS) algorithm. Digital images were converted to binary pixel maps by thresholding of the RGB (red-green-black colour model) filter. Collagen is converted to white (logical unity) pixels and non-collagen components to black (logical zero) pixels. Binary pixel maps were then evaluated using BinaryDirections software. The final orientation of collagen

fibers was obtained as the average of the results from four histological images. The software BinaryDirections and RoLS algorithm is described in details in [8,9].

Constitutive Model. The hyperelastic Holzapfel-Gasser-Ogden (HGO) nonlinear anisotropic constitutive model [10] was used to fit the experimental data. The strain energy density function is expressed by Eq. 6.

$$W = \frac{C}{2}(I_1 - 3) + \frac{k_1}{k_2}\left(e^{k_2(I_4-1)^2} - 1\right) \tag{6}$$

In Eq. 6 C and k_1 are stress-like parameters, k_2 is dimensionless parameter. I_1 is the first invariant of the right Cauchy-Green strain tensor and I_4 is additional invariant arising from material anisotropy and has the meaning of square of the stretch in preferred (fiber) direction. I_1 and I_4 are defined in Eq. 7 and Eq. 8.

$$I_1 = \lambda_r^2 + \lambda_t^2 + \lambda_z^2 \tag{7}$$

$$I_4 = \lambda_t^2 \cos^2\beta + \lambda_z^2 \sin^2\beta \tag{8}$$

In Eq. 8 β defines orientation of preferred direction within the material measured from circumferential axis of the tube – in our specific case this orientation is the collagen fibre orientation approximation. According to [10], the stress in the sample is then computed using W via equations Eq. 9, 10, 11.

$$\sigma_{rr}^{MOD} = \lambda_r \frac{\partial W}{\partial \lambda_r} - p \tag{9}$$

$$\sigma_{tt}^{MOD} = \lambda_t \frac{\partial W}{\partial \lambda_t} - p \tag{10}$$

$$\sigma_{zz}^{MOD} = \lambda_z \frac{\partial W}{\partial \lambda_z} - p \tag{11}$$

Here p is the Lagrange multiplier which plays a role of the reaction to incompressibility constrain. The Eq. 9 was combined with σ_{rr}=-$P/2$ (radial equilibrium equation) and then used to eliminate p. p is substituted into Eq. 10 and Eq. 11.

Objective function Q in Eq. 12 was minimized and the constitutive parameters of the model were calculated in Maple 16 (Maplesoft, Canada).

$$Q = \sum_{\substack{i=t,z \\ k=1..n}} \left(\sigma_{ii}^{EXP} - \sigma_{ii}^{MOD}\right)_k^2 \tag{12}$$

Results

The experimental data and computed model stress-stretch (σ-λ) curves for inflation-extension test are plotted in Fig. 3. The parameters of constitutive model obtained from regression analysis are listed in Table 2. Parameter β representing type I collagen fibres orientation was determined to be β = 0.71 rad (40.7°). The resulting polar-plot of collagen orientation determined using software BinaryDirections is shown in Fig. 4. Collagen orientation angle β obtained from histological analysis was β = 0.65±0.10 rad (37±6°), (mean±SD).

Table 2 Parameters of constitutive model and angle of collagen fibres in the wall

Constitutive model parameters				*Collagen orientation angle (histology)*
C [kPa]	k_1 [kPa]	k_2 [-]	β [rad]	β [rad]
2.50	10.46	32.88	0.71	0.65±0.10

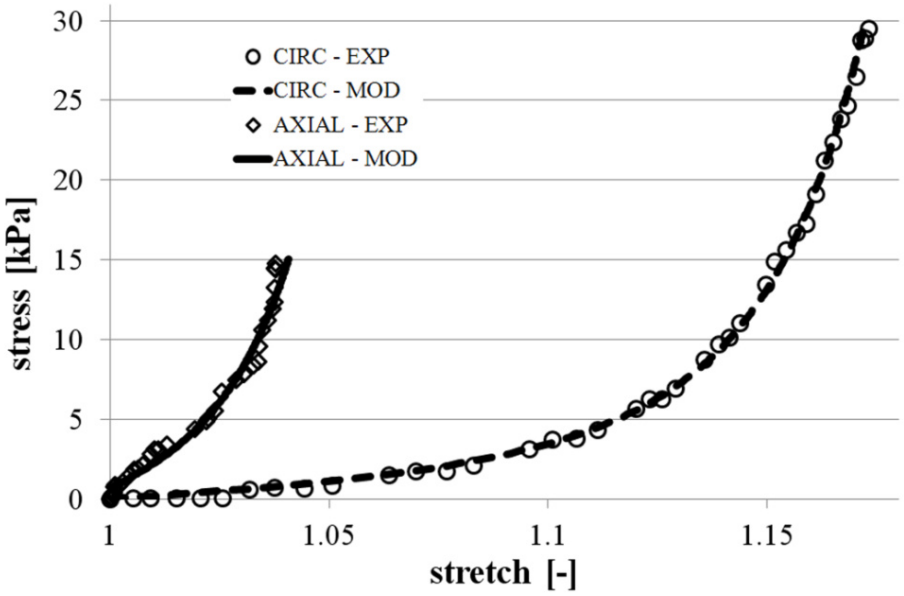

Fig. 3 The experimental data and computed model curves for the sample of the vein. CIRC and AXIAL denote circumferential and longitudinal direction, EXP and MOD denote experimental and model data.

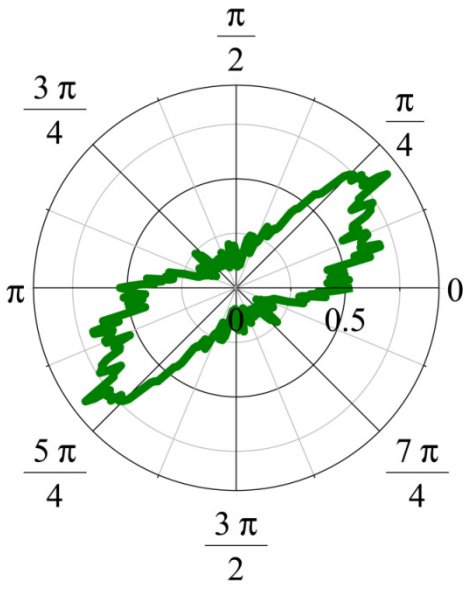

Fig. 4 Empirical probability density function of orientations of collagen fibres within the vein wall.

Discussion

The experimental data were fitted by anisotropic HGO constitutive model to obtain material parameters. Especially interesting parameter for us was the parameter β which corresponds to the preferred direction within the material – usually interpreted as the direction of collagen fibres. This parameter of the constitutive model was compared with the histology analysis of collagen orientation in the vein wall. It was found that collagen fibres orientation distribution (Fig. 4) has one maximum. For the tube it means that collagen fibres are helically aligned at an angle approx. 37° from circumferential axis of the vein. Table 2 shows a good correlation between both values obtained from constitutive model and histology.

The limitation of this preliminary study is related to the number of tested specimens. Only one sample obtained from one donor was tested. More samples from donors of different age and pathology should be studied but constitutive modelling in combination with non-destructive histological methods promise a good insight into tissue wall mechanics and architecture.

Acknowledgement

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