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ANISOTROPY OF SOFT BIOLOGICAL TISSUES AND ITS CONSIDERATION IN CONSTITUTIVE MODELS OF ARTERIES

Burša J.[*1], Hrubanová A. [1], Skácel P.[***1], Fischer J.[****1], Turčanová M.[*****1], Lisický O.[******1]**

Abstract: *Biomechanical computational models used more and more in medical research as well as in clinical applications need mathematical description of soft tissues with their very complex mechanical behaviour. They show large deformations with stress softening during cyclic loading, being moreover time-dependent due to their viscoelasticity, anisotropic due to fibrous structure and dependent on tissue excitation in case of muscular tissues, including smooth muscle cells in arterial walls. They are capable to adapt to the acting load (tissue remodelation) and to self-repair (healing) in case of damage or failure (rupture of fibres, etc.) This paper deals with constitutive description of passive elastic response of soft tissues to the acting load and with the ways, how the anisotropic structure of the tissue can be reflected. For this purpose, the spatial arrangement of fibres in the tissue needs to be detected and mathematically described. Lack of data on fibre arrangement in soft tissues and their interpatient variability is a major limiting factor for anisotropic constitutive description of soft tissues. Some problems related to transformation of results of mechanical tests and histological analyses of arterial tissues into parameters applicable in constitutive models are addressed as well.*

Keywords: Anisotropy, Mechanical properties, Hyperelasticity, Soft tissue, Collagen fibre.

1. Introduction

Biomechanical computational models gain importance not only in medical research but also in clinical applications, especially in orthopaedy and other surgical branches, e.g. cardio-vascular. Although hard tissues (bones) may be also anisotropic due to their trabecular structure, in most situations they remain linear elastic and thus can be described (with exception of time development of their properties) in a similar manner like technical composite materials. In contrast, soft tissues exhibit large deformations and thus are more difficult for constitutive description, for which hyperelastic models are used most frequently. In addition, their time dependent behaviour can be described using visco-hyperelastic models (see Fig. 1) or models of tissue remodelation (healing), and another category of models considers active response of some tissues (muscle contraction) or continuum damage mechanics approaches for stress softening effects. Although the time dependence of soft tissue properties may become significant (for instance, aorta rupture under extremely high strain rates at car accidents), this paper remains limited to hyperelastic behaviour of soft tissues, denoted often as pseudo-elastic. Collagen as dominant protein in human body with its fibrous structure may induce high anisotropy of soft tissues in dependence on fibre arrangement – especially on their directional distribution. Both isotropic and anisotropic hyperelastic models are used for their constitutive description, for instance in computational models predicting rupture of arterial aneurysms or evaluating vulnerability of atheromas. Their overview, analysis and comparison are presented in this paper.

[1] Inst. Solid Mechanics, Mechatronics and Biomechanics, Brno University of Technology, Technická 2896/2, 61669 Brno; CZ
[*] Prof. Ing. Jiří Burša, PhD.; bursa@fme.vutbr.cz
[**] Ing. Anna Hrubanová; 191418@vutbr.cz
[***] Ing. Pavel Skácel, PhD.; skacy@email.cz
[****] Ing. Jiří Fischer; 182495@vutbr.cz
[*****] Ing. Michaela Turčanová; turcanova@fme.vutbr.cz
[******] Ing. Ondřej Lisický, PhD.; o.lisicky@seznam.cz

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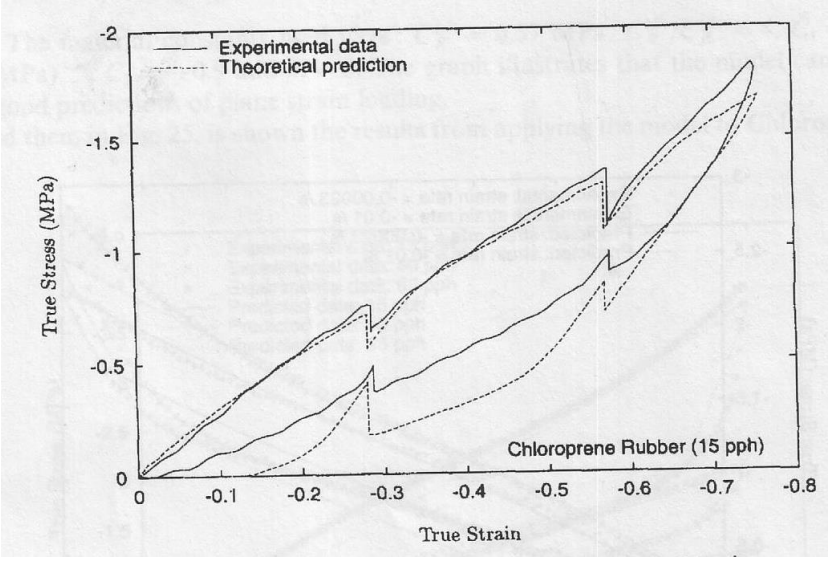

Fig. 1: Example of visco-hyperelastic behavior of elastomer in uniaxial compression test and its simulation using Bergström-Boyce model. The load-unload process is interrupted four times for 30 s to enable stress relaxation (from Bergström & Boyce 1998).

2. Mechanical properties of soft tissues and their testing

Similar to elastomers, uniaxial tension tests are sometimes not sufficient for description of multiaxial response of soft tissues, even if realized in several directions to cover the tissue anisotropy. In addition, biaxial tests are recommended to be applied especially with tissues being under biaxial load under physiological conditions (arterial walls, different membranes etc.). Moreover, triaxial load related closely to tissue (in)compressibility represents a specific issue.

2.1. Uniaxial testing

Stress-strain responses of soft tissues such as skin, arterial wall, diaphragm, fascia, endo- epi- and pericardium, etc. are highly non-linear exhibiting a progressive strain stiffening (see fig. 2c). It is generally acknowledged that the higher stiffness of arterial wall under supraphysiological pressure (and strain) is caused by straightening of wavy collagen fibres. Thus the response under low strains (in most of the physiological range of loads) is dominated by less oriented tissue components (elastin and also smooth muscle cells in some arteries); the straightened and very stiff collagen fibres dominate under higher (supraphysiological) loads and their orientation induces more significant anisotropy (see fig. 2). As biaxial tests require larger specimens which are mostly not available at arteries, uniaxial tension tests in different directions represent basic approach in mechanical testing of arteries. It is worth to mention that from a loading curve under constant strain rate we cannot detect any non-elastic effects; for large arteries, (pseudo)hyperelasticity is mostly assumed but several preconditioning cycles are applied before testing to saturate the stress-strain response and minimize the non-elastic effects.

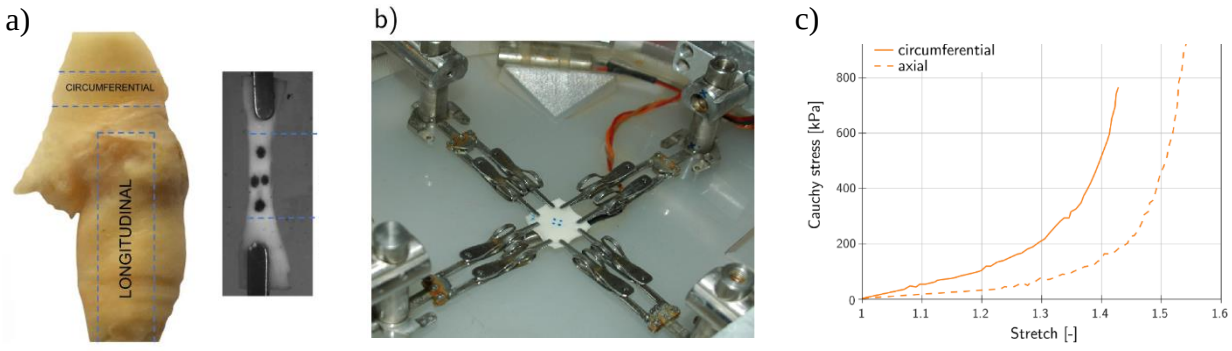

Fig. 2: Arterial specimens and their uniaxial (a) and biaxial (b) testing (Lisický et al. 2021). Stretch-stress response of arterial wall in biaxial testing (c).

2.2. Biaxial testing

As arterial samples are not large enough for cruciform specimens, two basic types of biaxial tests may be applied: biaxial tests with planar specimens or inflation of tubular specimens. Biaxial tests of planar specimens require unconstrained transversal contraction of the specimen, thus the load is applied via a set of either hooks or narrow clamps (see fig. 2b). Loads in both directions can be set independently and can be displacement controlled, force controlled or strain controlled. It is worth to mention that equibiaxial strain does not correspond to equibiaxial stress and vice versa. Inflation of tubular specimens is more physiological but applies more strict assumptions on homogeneity of stresses, as well as of the tissue specimen itself. Impact of fibre straightening on tissue stiffness is illustrated in Fig. 3.

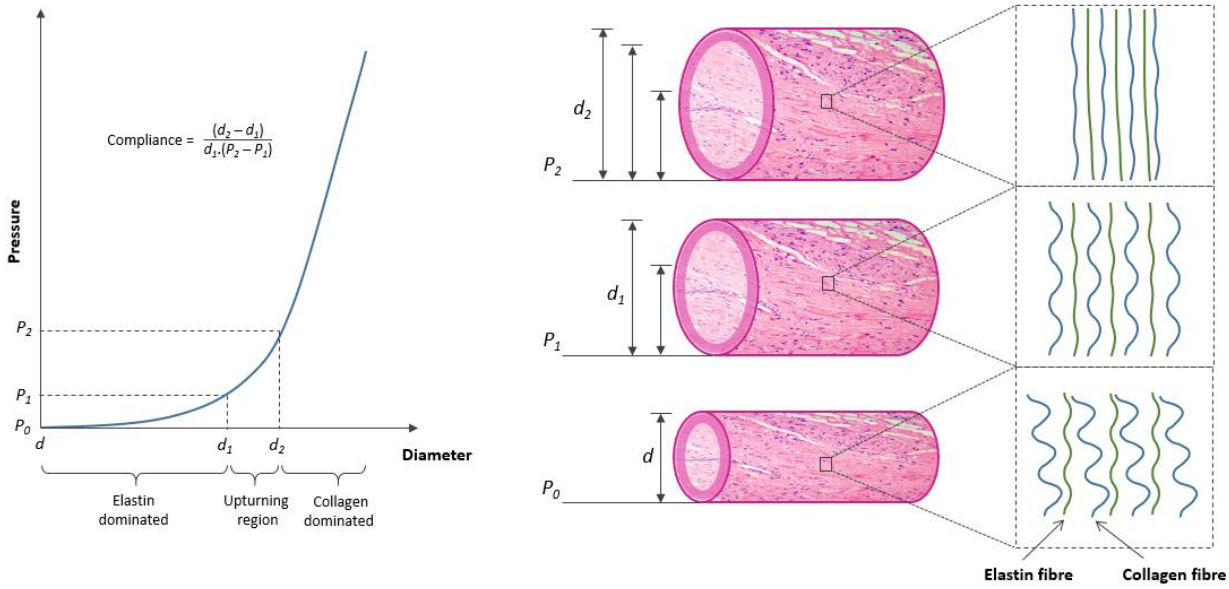

Fig. 3: Straightening of circumferential collagen fibres and their contribution to tissue stiffness in different parts of the response in the inflation test of a tubular specimen. Modified from Singh et al. (2015).

2.3. Testing of tissue compressibility

As many constitutive models assume tissue incompressibility this issue is decisive for their formulation and suitable choice. Compressibility of arterial tissue has been investigated for decades with contradictory results. This may be due to different testing methods, size dependence and other factors. Skácel & Burša (2022) investigated the arterial tissue compressibility together with its anisotropy under uniaxial tension; they exploited accurate (under optical microscope) 3D evaluation of all the 3 normal strains components and all Poisson's ratios. The results show tissue volumetric compressibility in the order of lower units of percents and uniquely reject auxetic behaviour of the tissue (i.e. transversal out-of-plane expansion under uniaxial extension). These results falsify predictions of some frequently used anisotropic constitutive models based on collagen fibre structure as analysed below in detail.

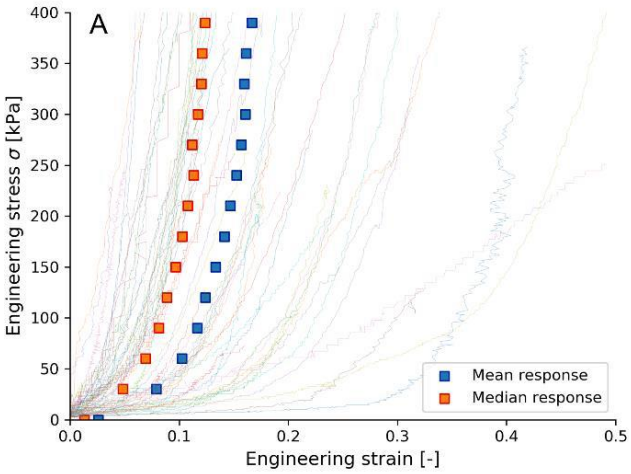

Fig. 4: Uniaxial stress-strain responses of some 100 specimens of human carotid arteries (Lisický et al. 2021a).

2.4. Dispersion of experimental data

Large dispersion and variability of all the properties is a typical feature of living, especially human tissues. Thus the way of obtaining average (mean population) responses (stress-strain curves) is not only very important but also rather challenging. Especially transformation of the responses into constants of a phenomenological model and calculation of their average values is rather risky (although frequently done) and may bring false results because these constants have no clear physical meaning and each response may be approximated using different combinations of constants of the chosen model. Moreover, it is rather usual with living tissues that the standard deviation is comparable in magnitude with the mean, even for material parameters being positive by principle (a.o., Young modulus, tensile strength). Whenever this is the case, statistical analysis of the data must include tests of data normality. Fig. 4 illustrates the difference between mean and median values of stress-strain curves of human carotid arteries. Here the asymmetric distributions of strains calculated for the chosen stress levels show evidently a large dispersion and should be represented as mean and interquartile range (Lisický et al. 2021b).

3. Constitutive models of soft tissues

Similarly to elastomers, isotropic hyperelastic models of soft tissues are mostly formulated on the basis of strain energy density function (SEDF) expressed by means of invariants of some (mostly Cauchy-Green) deformation tensor. All models below are presented as incompressible. As all the needed mechanical tests can seldom be realized with biological tissues and more complex phenomenological models show a poor predictive capability, Yeoh polynomial model is preferred among them:

$$W = \sum_{i=1}^{n} c_{i0}\left(I_1 - 3\right)^i$$

where I_1 is the first invariant of Cauchy-Green deformation tensor and c_{i0} [Pa] are material parameters, specifically c_{10} is related to initial modulus of elasticity and the others to the strain stiffening.

Orthotropic models exploiting exponential forms of SEDF expressed by means of individual components of deformation tensor in material coordinates are called Fung-type models. For instance, Chuong & Fung (1983) used the following formulation:

$$W = \frac{c}{2}\left[e^{Q} - 1\right] \quad \text{where} \quad Q = c_1\varepsilon_t^2 + c_2\varepsilon_z^2 + 2c_3\varepsilon_t\varepsilon_z$$

and c[Pa], c_i [-] are material parameters; ε_i are components of Green-Lagrange strain tensor.

3.1. Structure-based models

First structure based constitutive models of soft tissues considering the orientation of collagen fibres were introduced in 1980s. The **microfibre model** (Lanir 1983) integrates contributions of all structural components to the strain energy of a fibre composite and introduces some additional (pseudo)invariants of the deformation tensor for this purpose. Applications of this model were rare and are still rather challenging and time consuming even with advanced computers because it requires additional numerical integration (over all spatial directions) to be done at each integration point of the material. However, simplifications introduced in and after 2000 and huge expansion of computer capabilities enabled their broad application.

Deviatoric SEDF W_{dev} is decomposed into the isotropic contribution of matrix W_{iso} and anisotropic contribution of fibres W_{aniso}.

$$W_{dev} = W_{iso}\left(C\right) + W_{aniso}\left(C, A, B\right)$$

For the isotropic part W_{iso}, any suitable hyperelastic model can be used (neo-Hooke, Yeoh, ….), while for the anisotropic part W_{aniso} exponential models are introduced most frequently. A and B represent here the so called „structural tensors“ related to directions of fibres. They were introduced in the **HGO model** (Holzapfel et al., 2000) on the basis of an assumption on two perfectly aligned helical fibre families, being mechanically equivalent and symmetric with respect to the circumferential direction of the artery. This model exploits only two of the additional invariants representing stretches of both fibre families and is formulated as follows:

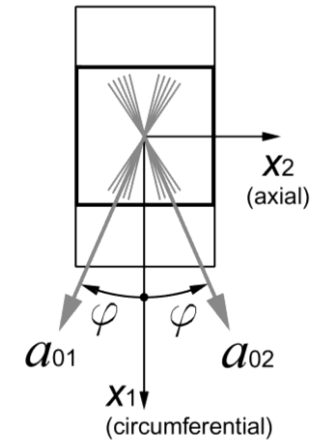

Fig. 5: Two families of dispersed fibres as assumed in the GOH model (Gasser et al. 2006).

$$W = \frac{\mu}{2}(I_1 - 3) + \frac{k_1}{2k_2}\sum_{i=4,6}\left(e^{k_2(I_i-1)^2} - 1\right)$$

Here μ*[Pa]* is shear modulus of the isotropic part (matrix), k_1*[Pa]*, k_2*[-]* are material parameters and I_4, I_6 are (pseudo)invariants of the right Cauchy-Green deformation tensor and A, B structural tensors. The fibres are considered to bear the load under extension only.

As collagen fibre directions are not perfectly aligned in soft tissues, Gasser et al (2006) introduced dispersion of fibre directions into this model through the so called generalized structural tensor (GST). This allowed to avoid angular integration (AI) of SEDF contributions (throughout all the fibre directions). Although this GST approach cannot sufficiently approximate the AI approach (as demonstrated in Skacel & Bursa 2014), it is computationally much more efficient and hence relatively popular. The SEDF of this **GOH model** is as follows:

$$W_{aniso(I_4,I_6)} = \frac{k_1}{k_2}\sum_{i=1}^{2}\left\{e^{\left[k_2(\kappa I_1 + (1-3\kappa)I_{4i} - 1)^2\right]} - 1\right\}$$

where κ represents a dispersion parameter of the fibres; $\boldsymbol{\kappa = 1/3}$ holds for isotropic distribution of fibres while $\boldsymbol{\kappa = 0}$ for perfectly aligned fibres; such value reduces the GOH formula into the HGO model. The introduction of structure tensor causes that all fibres of a family are considered to correspond to its dominant direction. Consequently, the inaccuracy of the model increases with increasing dispersion of fibre directions in each family.

Although the above two models consider collagen fibre orientation, they still describe the strain stiffening of fibres phenomenologically using an exponential function. Martufi & Gasser (2011) considered the background of this effect and introduced it into the Lanir type of model (AI approach). Following other scientists in the field, they considered triangular distribution of stochastically defined waviness λ of fibres (ratio of contour length of a fibre to the distance between its endpoints - see fig. 6). The resulting progressivity of fibre response is then induced by gradual straightening of single fibrils with individual waviness, as the additional structural parameter of the model.

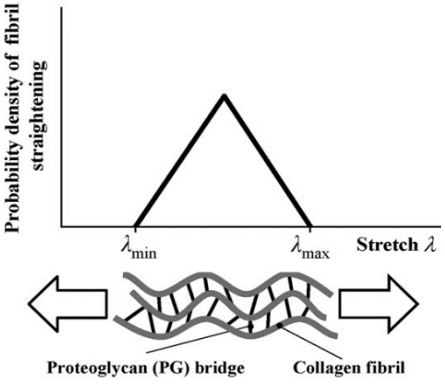

Fig. 6: Distribution of waviness λ within a fibre family (from Martufi & Gasser, 2011).

3.2. Contradictions of models with experiments

Unfortunately, the above models still suffer from a lack of experimental data on collagen fibre arrangement. If mechanical data are fitted without histological information (with the parameters of collagen fibre directions being calibrated from mechanical response), they give either nearly diagonal fibre arrangement (HGO model) or nearly isotropic fibre distribution (GOH model with κ=1/3), both being in contradiction to histology (Fischer et al., 2023). In contrast, if histological data on fibre arrangement are given, the models are not capable to reach an acceptable fit of mechanical data. The explanation can be found in fig. 2(c) where the responses in both directions show large strain stiffening but with circumferential orientation of collagen fibres (found most frequently in arteries) there is no tissue component to stiffen in the axial direction (neo-Hookean model of the matrix is without any stiffening). To overcome this discrepancy, **four-fibre family model** was introduced (Ferruzzi et al., 2011) by adding two more fibre families (circumferential and axial perfectly aligned fibres) to the HGO model with two original helical families. This model enables the needed strain stiffening in both axial and circumferential directions but has no histological substantiation. Similar results can be reached using **combination of Yeoh model with GOH**

model ensuring the isotropic strain stiffening and having all fibres close to the circumferential direction in accordance with histological results (Fischer et al., 2023).

Another limitation of these models is in their non-realistic prediction of transversal strains under uniaxial tension (Skácel & Burša, 2019). This effect is illustrated in Fig. 7 showing a specimen of arterial wall with two helical fibre families under uniaxial tension. Collagen fibres are stiffer by orders than the other components of arterial wall, thus they can be considered as rigid. The specimen is elongated by uniaxial stress σ, which is enabled only by rotation of the diagonal fibres resulting in negative transversal strain (in-plane component), potentially even larger in magnitude than the longitudinal strain. To compensate this in-plane contraction and simultaneously to keep the incompressibility of the matrix, the model induces large out-of-plane expansion resulting in a negative Poisson's ratio of the model. However, this behaviour was rejected by experiments of real arterial tissue (Skácel & Burša, 2022); that represents, in addition to histological results, another refutation of existence of two helical fibre families in arterial wall.

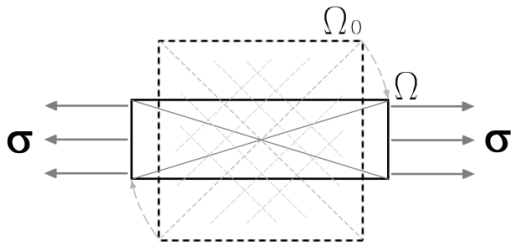

Fig. 7: Schematic demonstration of fibres rotation induced by tensile stresses; dashed and solid lines represent undeformed and deformed body Ωo and Ω, respectively.

3.3. Mathematical comparison of helical and wavy fibres in arteries

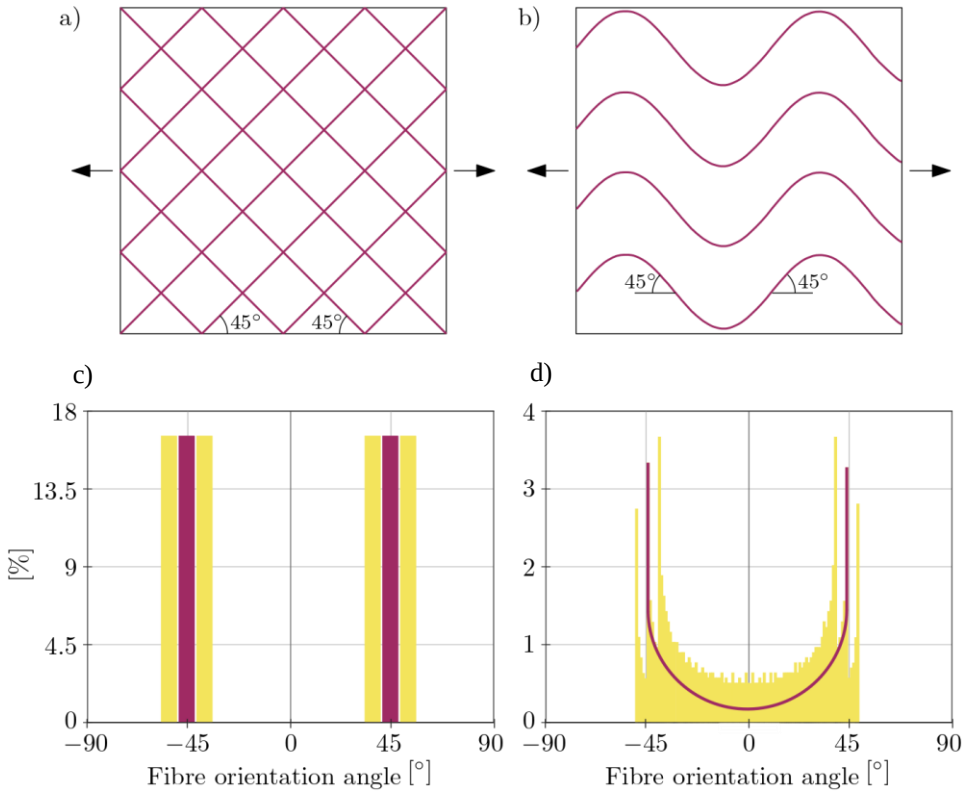

Fig. 8: The top row shows idealized arrangement of collagen fibres in arterial wall with two helical fibre families (of straight/waveless fibres) under ±45° (a) and one perfectly aligned family of wavy fibres with the same maximum angle of ±45° (b). The bottom row shows (in violet colour) the corresponding histograms with (c) being for helical fibres; they are completed (in yellow) with additional fibre families under the angles of ±40°and ±50°. The histogram of wavy fibres (d) reminds of real histograms of collagen fibres in arteries; evidently under uniaxial load, the two peaks of the histogram would get closer to the direction of load indicated by arrows in fig.(b). Modified from (Kratochvíl 2020).

In the mathematical models presented below we assume a perfectly compliant matrix and (similarly to Holzapfel et al. 2000; Gasser et al. 2006) zero bending stiffness of the fibres, thus they cannot bear any

load if not being straight and oriented in the direction of load. The models with two helical fibre families (a) and with one family of wavy fibres (b) are presented in Fig.8 for the same fibre angle of ±45°. Both of them give symmetric histograms, which would be hardly distinguishable if a significant dispersion existed., For the model (a) with straight and helical fibre families, however, the limit specimen stretch for fibre rotation towards the direction of load is $\lambda_0 = 1/\cos\alpha$, i.e. $\lambda_0=1.41$ for $\alpha=\pm45°$. In contrast, for the single family of sinusoidal fibres with the same local angles, the numerical integration along the curve length gives the limit stretch value for fibre straightness only $\lambda_0=1.22$ (see Kratochvíl 2020). The related histograms are completed with fibre angles of 40° and 50°, obtained typically when fitting the GOH or HGO models to experimental data without histological information on fibre directions (Haskett et al., 2010; Fischer et al., 2023; Schriefl et al., 2012). The limit stretches λ_0 for the angles of 40° and 50° are 1.31 and 1.56 for helical fibres and 1.16 and 1.29 for wavy fibres, respectively. If recalculated into engineering strains $\varepsilon_0 = \lambda_0-1$, the values for fibres arranged in two helical fibre families are approximately two times higher than for wavy fibres with the same maximum local angle. This is due the fact that only an effective portion (~50%) of contour length of wavy fibres undergoes reorientation during stretching of (globally aligned) fibres, in contrast to straight helical fibres, which undergo re-orientation in their whole length.

The models (a) and (b) in fig. 8 enable also some considerations on the tissue behaviour under biaxial extension. Under equibiaxial stretch, no fibre rotation is initiated in the model (a), the fibres are elongated (and bearing load) from the very beginning and there is no reason for a strain stiffening of their response. In contrast, the response of the model (b) is similar to uniaxial tension and the impact of the extension transversal to the global orientation of fibres depends on the affinity of deformation between the fibres and matrix. This type of behaviour corresponds much better to the real behaviour of arterial tissue under equibiaxial load (see fig. 2c).

4. Arrangement of collagen fibres

Fibre waviness, however, complicates critically evaluation of fibre directions. In fact, nearly all methods used for detection of collagen fibre directions investigate their local directions, while the constitutive models require their global orientation in the undeformed state of the tissue. As shown in fig. 8, for sinusoidal fibres the most frequent local directions are those most different from the global fibre direction. This discrepancy could be overcome by detection of fibre directions under load inducing their straightening. It was shown experimentally that the concentration parameters of fibre distribution increase with increasing fibre extension even in cases when their global re-orientation is avoided. Turčanová et al. (2023) have shown that the increase in the concentration parameter of fibre directions is similar under equibiaxial load and uniaxial load acting in the dominant fibre direction, while under perpendicular load it does not increase significantly. All this shows that fibre directions should be investigated together with their waviness. There are two approaches how to do it.

First, the fibre directions can be evaluated under load (see fig. 9). In this way, the fibre straightening ensures higher concentration parameter evaluated from their local orientations (see fig. 10 a)) and the fibres global orientation then tend to unimodal distribution. Under uniaxial load, however, the fibres rotate significantly and the evaluated dominant direction may differ from that in the unloaded state, required for the constitutive models (which may be, however, simply eliminated by pull-back operation of the resulting distribution). In contrast, no fibre rotation should occur under equibiaxial load (see fig. 9) but it is true under equibiaxal strain only, which does not correspond to equibiaxial stress. However, equibiaxal state of strain is more difficult to be reached because it requires in-time strain evaluation for the strain controlled feedback while equibiaxial tension tests are mostly stress controlled or displacement controlled and the strains are evaluated afterwards.

The second approach is applicable after having detected the contours of individual fibres (fibre tracking), for instance using confocal microscopy (Turčanová, 2023). Then the global fibre direction is given by vector connecting its endpoints. Unfortunately, this procedure has not been automated yet (for collagen fibres) and requires manual detection of each fibre. Thus the correct evaluation of parameters applicable in the constitutive models represents the most important challenge in this field.

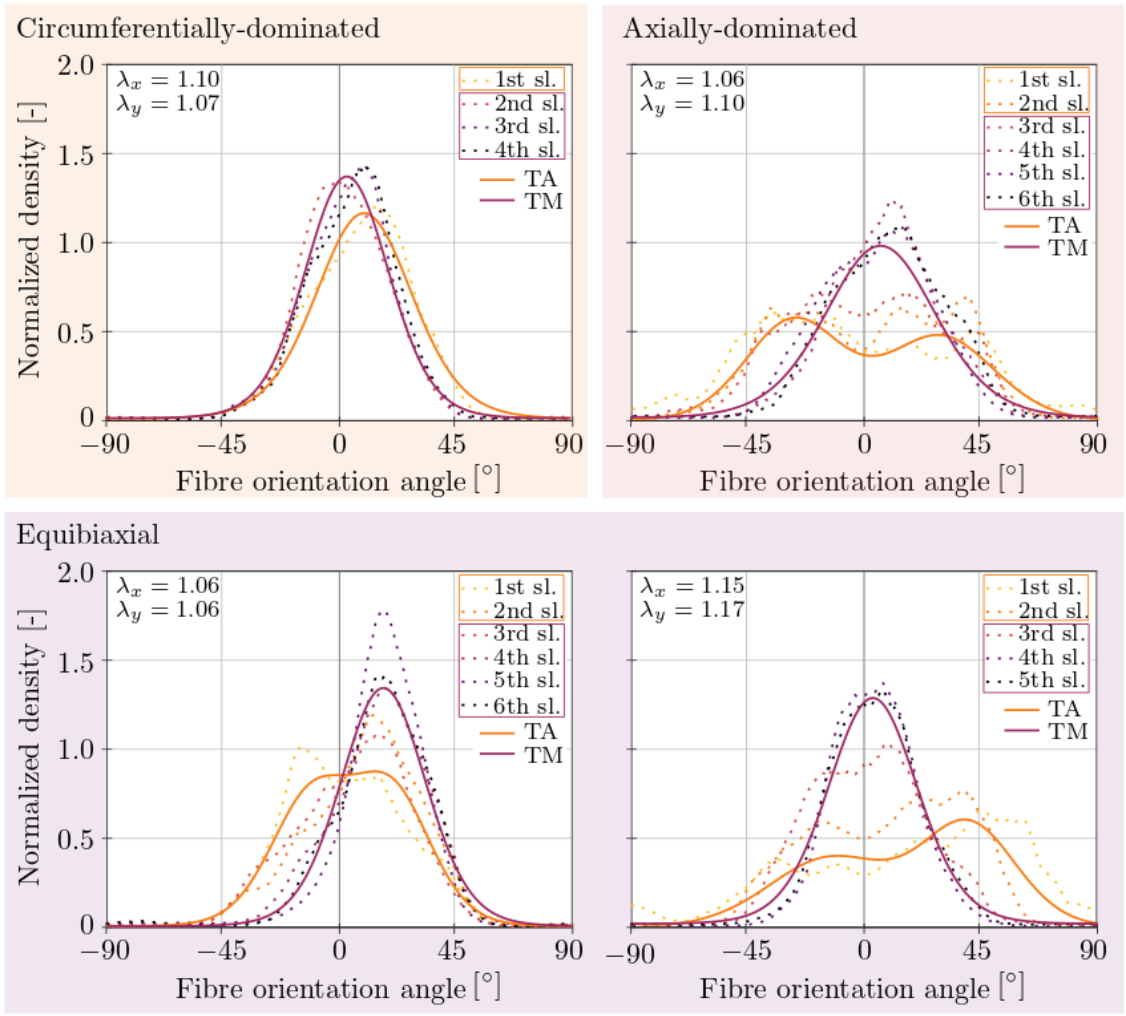

Fig. 9: Histograms of collagen fibre directions in porcine aorta under different biaxial loads. Raw histograms from all the evaluated sections are depicted as dots, the individual slices are ordered from the outer to the inner surface. The von Mises distribution functions (unimodal or bimodal) are presented as solid lines common for adventitia (orange) and media (purple) layers. 0° is circumferential direction, TM and TA mean media and adventitia layers, respectively.

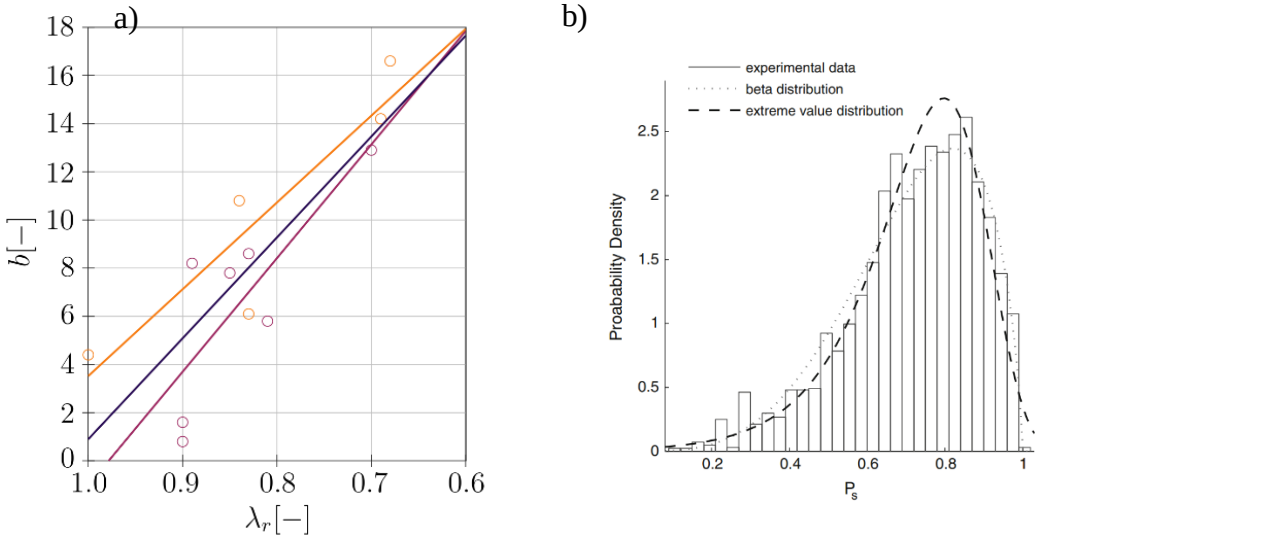

Fig. 10: (a) Dependence of the concentration parameter b on the radial stretch λ_r. Circles represent experimental results and are approximated with straight lines. Orange and violet colours represent two different experiments and the black line is their average. (b) Histogram of fibre straightness. From (Turčanová 2023).

5. Conclusion

Anisotropy of soft tissues may be significant. The advanced structure-based constitutive models enable us its description but under the following limitations:

- Knowledge on tissue structure is needed, especially on distribution of collagen fibre directions in the unloaded state.
- The models require knowledge of global directions of collagen fibres, which may be significantly different from the local directions detected by most of the applicable methods due to fibre waviness. Bimodal distributions of local fibre directions, may be misinterpreted as two fibre families while representing one family of wavy fibres.
- As the concentration parameters increase with extension of fibres and their decreasing waviness, the global fibre directions can be advantageously evaluated under uniaxial or biaxial extension. Nevertheless, they need to be recalculated back to the undeformed conditions needed for the constitutive models.
- Although distribution of fibre waviness is detectable using some of the up-to-date methods, there are hardly any attempts to transform these results into applicable constitutive parameters.
- Although anisotropy is often significant at individual human tissues, the inter-patient variability may be large and make the anisotropy statistically insignificant for mean population models.
- Comprehensive mechanical and histological testing can hardly be carried out non-invasively and without it the advantages of structure based constitutive models may get lost and isotropic models may become more effective.

Acknowledgement

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