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Mechanical characterization of bovine skeletal muscle under simple shear

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Abstract: The aim of this work is to investigate the mechanical behavior of skeletal muscle tissue subjected to simple shear. Simple shear tests were performed on bovine skeletal muscle samples obtained from a local regulated slaughterhouse. Specimens with muscle fibers oriented at 0° with respect to the applied shear load were tested. In the experimental procedure, the shear and lateral normal stress components, as well as the amount of shear, were simultaneously determined using a new shear test apparatus. The values of amount of shear were assessed using the digital image correlation method. Only positive Poynting effect, i.e., negative normal stress was observed when bovine skeletal muscles were sheared. This result indicates that the material expands laterally during the test. The strain-stiffening effect was also observed. The findings confirmed adequate reproducibility and reliability of the novel experimental protocol for measuring shear and normal stresses that arise on bovine skeletal muscle in simple shear. Vito model was used to verify the mechanical elastic behavior of the soft tissue. In general, a reasonable correlation was found between model predictions and measured data for shear stress. Therefore, the proposed measurement method may be a useful tool for evaluation of muscle stiffness/response. This contribution aims to investigate and develop new experimental techniques for skeletal muscle tissue that allows obtaining material properties under simple shear.

Keywords: Skeletal muscle, Simple shear test, DIC method, Poynting effect.

INTRODUCTION

Recently, there has been growing interest in mechanical behavior of soft solids such as natural and synthetic rubbers and soft biological tissues. In general, these materials exhibit highly nonlinear behavior under large deformations. In biological soft tissues, high normal stresses may be developed during physiological response due to large shear strains that could potentially contribute to their injury (Balbi et al, 2019) or to human perception of surgical simulators (Misra et al, 2010). They are naturally made of a complex structure. Besides, there are challenges associated with attaching soft tissues to testing systems and achieving the boundary condition reliably (Jiang et al., 2020).

Simple shear and torsion tests have been employed to characterize mechanical properties of soft biological tissues. However, they are not sufficient due to the difficulties related to the implementation of shear tests, especially in biological soft tissues. Some theoretical studies have shown that, depending on the degree of anisotropy and the angle of orientation of the fibers, the normal stress required to maintain the simple shear state can be compressive or tensile, corresponding to classical (positive) and negative (reverse) Poynting effects, respectively. This effect has recently been researched for biomaterials and fibrous tissue (Murphy, 2013, Horgan and Murphy 2017a, 2017b).

Destrade et al. (2015) have developed research in which the positive Poynting effect was observed in porcine brain matter subjected to simple shear. They highlighted that the normal force data or another testing protocol are required to complete its material characterization when the material is under simple shear deformation. The authors emphasized that it is not simple to measure the normal force. On the other hand, the normal force produced by the positive Poynting effect was measured in porcine brain matter subjected to torsion (Balbi et al., 2019). Due to experimental limitations of the rheometer, the authors pointed out that there is a lack of shear devices capable of measuring the normal force. Thus, further experimental work on biotissues is clearly desirable and the important issue of normal stress remains controversial.

More recently, in the work developed by Sugerman et al. (2021), the negative Poynting effect was observed in a whole blood thrombus mimic subjected to simple shear. In addition, the effects of fiber-matrix interaction on the Poynting effect were investigated for simple shear (Horgan and Murphy (2021a)) and torsion (Horgan and Murphy (2021b)) of fibrous soft tissues. It was shown that the fiber-matrix interaction enhances both the positive and negative Poynting effects for simple shear while negative Poynting effect increase for torsion. These effects were considered on the modeling of the material.

A problem in formulating constitutive equations to describe the soft biological tissue behavior is the limited availability of experimental data. As shown, in most reported experiments, only the shear stress behavior of soft tissue has been determined. However, additional information is needed on the muscle's lateral normal stress.

For this reason, the purpose of the present work is to simultaneously determine the shear and normal stresses of bovine skeletal muscle using a new simple shear apparatus. The amount of shear is also assessed from displacement fields obtained by the digital image correlation method. Because the muscle behaves highly nonlinear due to the strain-stiffening and Poynting effects, the Vito model is employed to describe the shear and normal responses of the bovine skeletal muscle.

EXPERIMENTAL PROCEDURE

Each sample was cut of a bovine skeletal muscle obtained from a local regulated slaughterhouse. Prismatic samples of such muscle (70 mm x 55 mm x 10 mm) were extracted using a manual cutting process. Firstly, a cutting guide was manufactured from rectangular bars of aluminum to reduce the variations of the sample dimensions and facilitate the cutting operation, as shown in Fig. 1(a) and Fig. (b)). The sample cutting was performed using a sharp knife (Figure 1(c)). For each set of samples, the reinforcement was oriented at 0° related to the main sample dimension (applied shear force direction), as shown in Figure 2. After extraction, the samples were placed in a fridge (~ 4-5 °C) and kept hydrated until the beginning of the test. Time between sample cutting and testing was inferior to 8 h at most.

(a) **(b)** **(c)**

Figure 1 – Cutting guide and knife used to obtain the prismatic samples of bovine skeletal muscle.

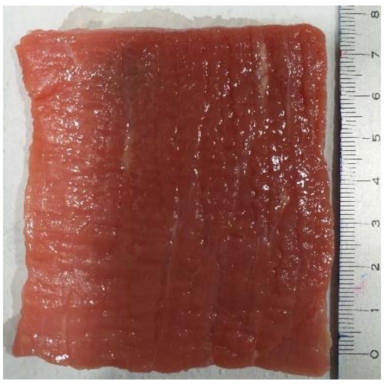


Figure 2 – Rectangular sample of bovine muscle with fibers oriented at 0° related to main dimension (~ 70 mm) (c).

The experimental arrangement was composed of the shear test apparatus (STA), which was coupled to a motorized testing machine, and the Digital Image Correlation (DIC) system, as illustrated in Fig. 3. In view of the difficulties associated with performing simple shear test in soft materials using conventional approaches, STA was developed to determine shear and normal stresses and shear strain simultaneously. More details on STA can be found in Araújo and

Nunes (2020). In the proposed arrangement, 1st and 2nd load cells measure the shear (F^S) and normal (F^N) forces, respectively. It is important to emphasize that the two support plates (holders) move parallel towards the applied load, keeping the distance (h) constant of 5 mm between them. Accordingly, the left plate was kept fixed while the right plate could move down in the vertical direction.

Each specimen with 8 mm of thickness was clamped in STA with an initial shear region of 70 mm (edge parallel to X_1-axis) × 5 mm (edge parallel to X_2-axis). Before testing, the dimensions of each sample were measured using a vernier caliper and recorded. Grips were covered in grade P80 sandpaper to enhance adherence. Also, glue was used to attach the samples to the grips. In the sequence, the holders were positioned and fastened by means of eight screws. To prevent tissue damage during gripping, the bolts were tightened using a manual tool, as this was found empirically to minimize both slippage (grips too loose) and tissue damage (grips too tight). Each specimen was tested only once since large strain level can lead to some permanent tissue deformation. Rashid et al. (2014) pointed out that this procedure is important to prevent the tissue from losing its stiffness and preventing dehydration (because of the viscoelastic nature of tissue) and thus contribute towards repeatability in the experimentation.

One set of five specimens of soft material oriented at 0º was subjected to simple shear. Quasi-static shear tests were carried out at constant crosshead displacement rate of 8 mm/min and at room temperature of approximately 25 °C. To improve the image matching procedure, all specimens were sprayed with black and white paints to attain a random speckle pattern. Before paint application, only remaining liquid was removed from the surface of the samples using absorbent paper in order to retain the sprayed paints. A CCD camera (Sony XCD-SX910) with a 10 X Zoom C-Mount lens set perpendicularly to the specimen was used for capturing images during tests. Images were post-processed using a DIC program to extract full-field displacements of the specimen and to evaluate the amount of shear (k).

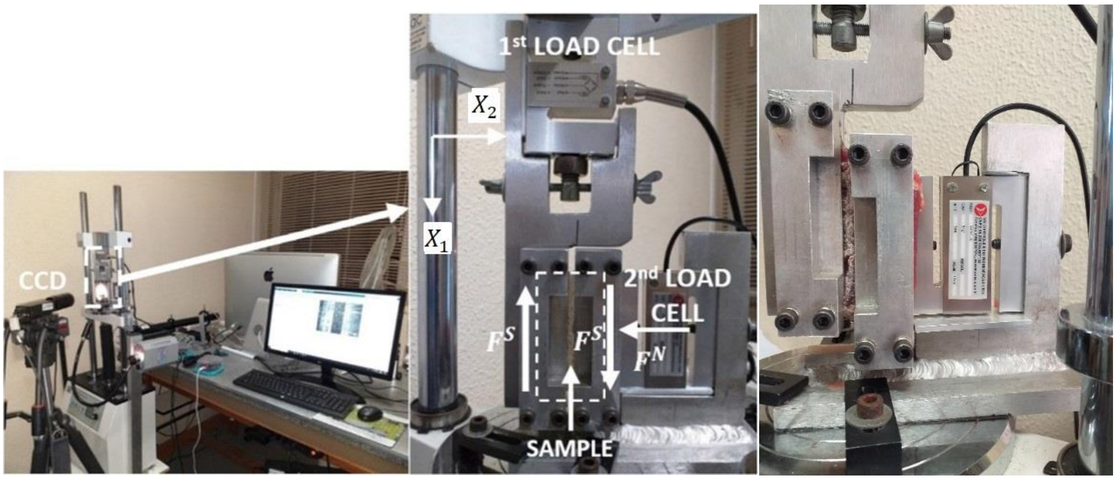


Figure 3 - Experimental arrangement of simple shear experiments on bovine skeletal muscle.

MODELING OF SIMPLE SHEAR

Fig. 4 illustrates schematically a rectangular soft tissue under simple shear. The lower edge is fixed while the upper edge is subjected to applied load (F^S) along the x_1-axis. It is known that most materials have a tendency to expand perpendicularly to the applied shear force, yielding a positive Poynting effect. For this reason, it is necessary to apply a negative normal force (F^N) in order to maintain the effective distance (h) between edges constant for attaining a simple shear state. Moreover, many rubber-like materials and soft biological tissues become stiffer as they are strained. This stiffness is not constant but increases when the material is strained (Horgan and Saccomandi, 2006; Jaspers et al., 2014; Schiavi et al., 2022). The body is assumed to be sheared by an amount k, and γ is the angle the sheared line makes with its original orientation. The current configuration (x_1, x_2, x_3) can be written as a function of the reference configuration (X_1, X_2, X_3) and amount of shear $k = tg\gamma$, considering h = 1, as follows:

$$x_1 = X_1 + kX_2, \; x_2 = X_2, \; x_3 = X_3 \tag{1}$$

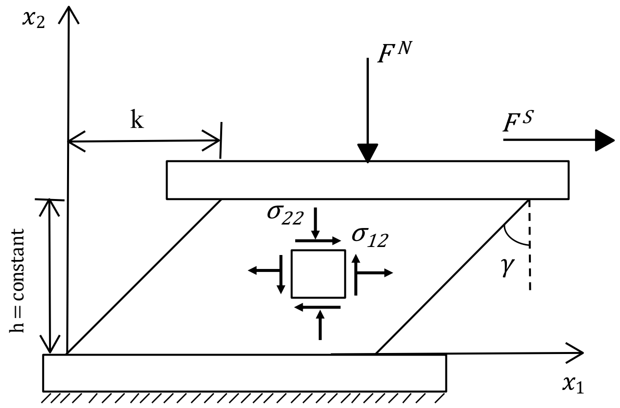


Figure 4 – A soft tissue under simple shear with negative normal force produced by the positive Poynting effect.

Using Equation (1), the deformation gradient tensor for simple shear can be expressed as

$$\boldsymbol{F} = \frac{\partial \boldsymbol{x}}{\partial \boldsymbol{X}} = \begin{bmatrix} 1 & k & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{2}$$

In this case, the left Cauchy-Green strain tensor is written as

$$\boldsymbol{B} = \boldsymbol{F}\boldsymbol{F}^{\boldsymbol{T}} = \begin{bmatrix} k^2+1 & k & 0 \\ k & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{4}$$

and the principal invariants of $\boldsymbol{B}$ are given by

$$\begin{gathered} I_1 = tr[\boldsymbol{B}] = k^2 + 3 \\ I_2 = 1/2[(tr\boldsymbol{B})^{\boldsymbol{2}} - tr(\boldsymbol{B})^{\boldsymbol{2}}] = k^2 + 3 \\ I_3 = 1 \end{gathered} \tag{5}$$

In general, for describing the mechanical behavior of soft biological tissues, a strain-energy density $W = W(I_1, I_2)$ that depends on the invariants (I_1 and I_2) is commonly considered. In this case, the corresponding Cauchy stress tensor can be given by (Rivlin, 1948; Nunes and Moreira, 2013)

$$\boldsymbol{\sigma} = -p\boldsymbol{I} + 2\left[\frac{\partial W}{\partial I_1}\boldsymbol{B} - \frac{\partial W}{\partial I_2}\boldsymbol{B}^{-1}\right], \tag{6}$$

where $\boldsymbol{B}$ is the left Cauchy–Green deformation tensor, $\boldsymbol{I}$ is the identity tensor and p is the arbitrary hydrostatic stress.

The stress components for simple shear deformation are schematically shown in Fig. 4. By using Equation (6), they can be

$$\sigma_{11} = 2k^2\frac{\partial W}{\partial I_1},\ \ \sigma_{22} = -2k^2\frac{\partial W}{\partial I_2},\ \ \sigma_{12} = 2k\left(\frac{\partial W}{\partial I_1} + \frac{\partial W}{\partial I_2}\right), \sigma_{13} = \sigma_{23} = 0, \sigma_{33} = 0 \tag{7}$$

A model with reasonably accurate prediction is essential to capture the strain-stiffening and Poynting effects that are observed on the experimental tests as the shear strain increases for the skeletal muscle. For some soft biomaterials, an exponential dependence on the first invariant is well known to assess the predominant stiffening effect as occur for Fung–Demiray (FD) model. A modification of this model that depends on the first and second invariants was proposed by Vito in order to reflect the Poynting effect (See Horgan and Smayda, 2015). In this work, the Vito model is considered. It is given as

$$W = \frac{\mu}{2b}\left[e^{b[\alpha(I_1-3)+(1-\alpha)(I_2-3)]} - 1\right] \tag{8}$$

where μ is the shear modulus for infinitesimal deformations, b is the non-dimensional positive constant provides a measure of the strain-stiffening characteristic of soft biomaterials and α is the non-dimensional parameter such that $0 < \alpha \leq 1$.

Since the observed Poynting effect was positive, *i.e.*, the specimen expands laterally, from macroscopic scale, it is assumed the skeletal muscle as isotropic since the matrix phase plays a significant role in the occurrence in this phenomenon during the simple shear. Thus, the strain-energy function, Equation (8), is dependent of the I_1 and I_2 invariants. It is important to mention that this behavior is opposite from that developed by transversely isotropic biological soft tissues, in which one family of parallel fibers is considered and the fibers react in tensile during the simple shear leading to a negative Poynting effect (Murphy, 2013, Horgan and Murphy 2017a, 2017b). Substituting Equation (8) into Equation (7), yields shear and normal stresses components:

$$\sigma_{12} = \mu k e^{bk^2},$$

$$\sigma_{22} = -\mu k^2 e^{bk^2}(1-\alpha) \quad (9)$$

In fact, the lateral normal stress is negative due to positive Poynting effect predicted by this model.

For infinitesimal deformations,

$$\sigma_{12} = \mu k \, , \, \sigma_{22} = 0 \quad (10)$$

This result is according to linear theory in which normal stresses are not produced during simple shear.

RESULTS AND DISCUSSION

Shear and normal stresses were experimentally obtained considering the shear (F^S) and normal (F^N) loads and the cross-sectional area of each specimen (70 mm x 8 mm) using the proposed STA. The values of amount of shear (k) were evaluated using the displacement fields of a small zone of the specimen, according to Equation (1). The average of all shear and normal stresses values in each sample was calculated. The mean shear and normal stresses as a function of amount of shear (k) for skeletal bovine tissue with fibers oriented at 0° are illustrated in Fig. 5. The mean value and standard deviation were obtained from five different samples for each configuration. Fig. 5 demonstrates good repeatability of results.

Thus, reliable experimental data can be obtained from the device, by carefully selecting the sample thickness and performing calibration before the tests. The horizontal distance between the platens can be adjusted based on the dimension of test specimen and platens. The left grip remains fixed while the right grip moves down, thus causing simple shear in the muscle tissue at a constant strain rate. Although mounting and adjusting the samples in the testing device with defined fiber orientation was straightforward and performed with great care, small deviations from a perfect alignment of the samples were sometimes inevitable, e.g., due to slight variations within the samples. In addition, the specimen preparation protocol and mounting procedure on the testing mechanism are crucial in achieving consistency and repeatability in the experiments. Therefore, the STA is suitable for testing prismatic specimens under simple shear.

It can be clearly seen from Fig. 5(a) that the material presents a typical J-shape curve, and the stiffness increases significantly with increasing amount of shear, as expected, *i.e.*, displaying an exponential shear stress versus amount of shear relationship. Thus, the strain-stiffening effect is verified at large deformation.

Moreover, a decrease variation of the slope of shear response is observed in k = 0.4. This behavior can be attributed to damage effect, i.e., failure initiation from fibers, or sample slippage during large deformations.

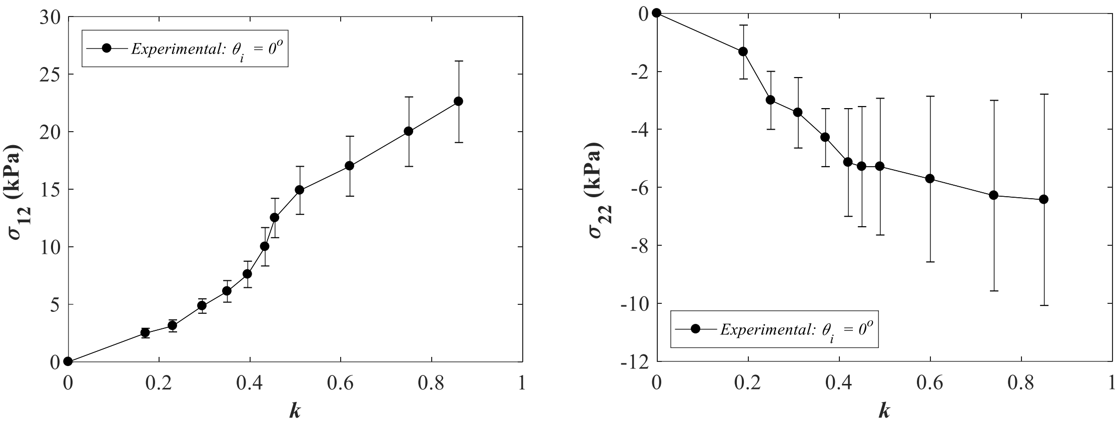


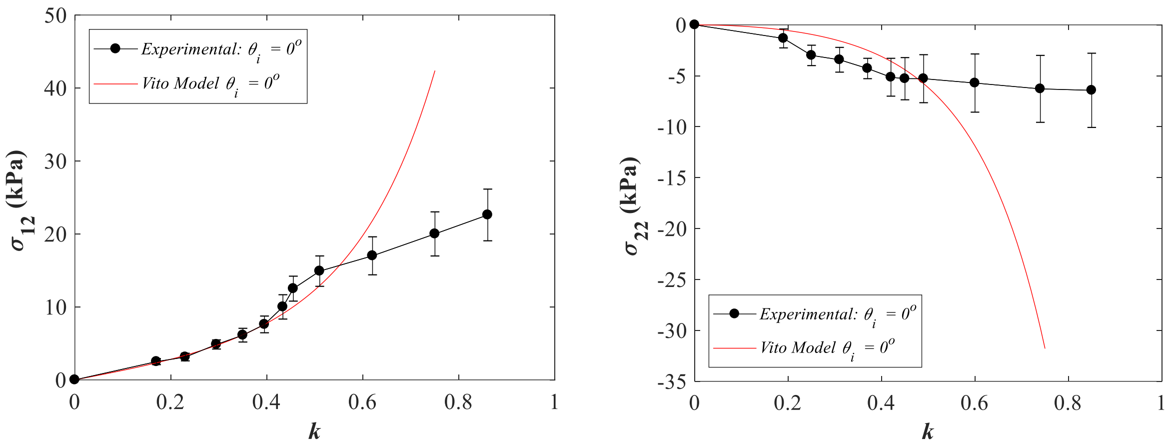
Figure 5 – Shear (a) and normal (b) stresses versus amount of shear of a bovine skeletal muscle for fibers at 0º: Experimental data.

In addition to shear stress, the lateral normal stresses were also measured. The effect of fiber orientation on the normal stress required to maintain the simple shear state is now examined thus determining the character of the Poynting effect. Fig. 5(b) shows the lateral normal stress (σ_{22}) as a function of the amount of shear for muscle specimens with fibers oriented at initial angles of 0º.

As can be seen from this figure, the normal stress is compressive (negative), resulting in a positive or classic Poynting effect, *e.g.*, the muscle specimen expands laterally during the shear. As previously mentioned, the failure of the tissue is also verified from $k \approx 0.4$ for normal stress curves. In this case, the matrix plays a significant role in the occurrence in this phenomenon during the simple shear. Note that, the positive Poynting effect is quite substantial, of the same order of magnitude of the applied shearing stress. It is important to remark that Figure 5(a) shows low deviation between the σ_{12} and k up until 0.4, but the same is not observed between σ_{22} and k on Figure 5(b). These results demonstrate the experimental difficulties related to quantify the normal stress. This discrepancy can probably be accounted for errors in experiments that can arise from many sources, including specimen slippage, measurement of the normal force that is perpendicular to applied shear load, pre-loading conditions, the natural anisotropy of the material, etc.

After damage, the soft skeletal muscles under shear do not exhibit well-behaved stress-k curves. For this reason, the study has concentrated on mechanical behavior up to $k \approx 0.4$. In Fig. 5 (b), the normal stress response is nonlinear in this range of shear strain. Therefore, these results can be used to verify the model previously presented in Section 3.

Since the Poynting effect is not known for this tissue, the Vito model was considered to represent the observed experimental behavior, which is based on the theory of nonlinear elasticity for incompressible isotropic materials. The first step is to run the curve-fitting exercise. Parameters of the constitutive model were obtained by fitting to shear stress - k curve data. The shear modulus (μ) and the stiffening parameter ($b > 0$) were estimated by fitting the equation (9a) to the shear stress- k curve taking values: 12.72 $\pm$1.625 kPa and 2.65$\pm$1.004. Fig.6 (a) and Fig.6 (b) show the measured data and fitted model for shear and normal stresses, respectively. The experimental results are the same of Fig. 5. Note that, the model predicts the strain-stiffening effect as shown in Fig. 6(a).



(a) (b)

Figure 6 – Comparison between experimental data and predictive model for skeletal muscle with fibers at 0º: shear (a) and normal (b) stresses.

Finally, in Fig. 6 (b), model predictions of the normal stress are compared with measured results for muscle with fibers oriented at 0°. The same parameters (μ) and (b) estimated previously were considered in Equation (9.b) to obtain the normal stress. The parameter (α) was considered at bound ($\alpha \rightarrow 0$). Note from this figure that, a reasonable agreement between model predictions and experimental data is only attained for values of k up to 0.1. In addition, there is a discrepancy between measured and expected results at large shear strain. These results indicate that the anisotropy likely may be taken into account for simple shearing of such tissue. Therefore, predictions using others constitutive models will be required to capture more accurately the effect of fiber-matrix interaction and fiber orientation on the positive Poynting effect for simple shear of bovine skeletal muscle.

CONCLUSIONS

In this study, the shear and normal stresses of bovine skeletal muscle under simple shear were investigated. A new simple shear apparatus was used to simultaneously measure shear and normal stresses on composite specimens with fibers oriented at 0º with respect to the applied shear load. The proposed measurement method constituted a useful tool for evaluation of muscle stiffness and assessing the shear and normal response of soft tissues and quantify the Poynting effect under simple shear. Negative normal stress was induced by the initial orientation of fibers, yielding a positive Poynting effect for this configuration. The Vito model were used to describe the tissue responses. In general, a reasonable correlation between model predictions and measured data was found for shear stress, but the discrepancy increases significantly for normal stresses at large deformations. Besides, the data may, therefore, be used to establish more accurate constitutive models to predict the material behavior.

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REFERENCES

Araújo, F.S. and Nunes, L.C.S., 2020, "Experimental study of the Poynting effect in a soft unidirectional fiber-reinforced material under simple shear," Soft Matter, Vol.16, pp. 7950-7957, https://doi.org/10.1039/D0SM00745E.

Balbi, V., Trotta, A., Destrade, M. and Annaidh, A.N, "Poynting effect of brain matter in torsion", Soft Matter, 2019, Vol.15, pp. 5147-5153.

Destrade, M., Gilchrist, M.D., Murphy, J.G., Rashid, B. and Saccomandi, G., 2015, "Extreme softness of brain matter in simple shear", Int. J. Non-Linear Mech, Vol.75, pp. 54-58.

Horgan, C.O. and Murphy, J.G., 2017a, "Poynting and reverse Poynting effects in soft materials", Soft Matter, Vol.13, pp. 4916-4923, https://doi.org/10.1039/C7SM00992E.

Horgan, C.O. and Murphy, J.G., 2017b, "Fiber orientation effects in simple shearing of fibrous soft tissues", Journal of Biomechanics, Vol. 64, pp. 131-135, https://doi.org/10.1016/j.jbiomech.2017.09.018.

Horgan, C.O. and Murphy, J.G., 2021a, "Fiber-Matrix Interaction and Fiber Orientation in Simple Shearing of Fibrous Soft Tissues", Journal of Elasticity, Springer Nature B.V, https://doi.org/10.1007/s10659-021-09847-3.

Horgan, C.O. and Murphy, J.G., 2021b, "The effect of fiber-matrix interaction on the Poynting effect for torsion of fibrous soft biomaterials", Journal of the mechanical behavior of biomedical materials. Vol. 118, pp. 104410.

Horgan, C.O. and Saccomandi. G., 2006. "Phenomenological hyperelastic strain-stiffening constitutive models for rubber", Rubber Chemistry and Technology, Vol. 79, pp. 152-169.

Horgan, C.O. and Smayda, M.G., 2012, "The importance of the second strain invariant in the constitutive modeling of elastomers and soft biomaterials", Mechanics of Materials, Vol. 51, pp. 43-52.

Jaspers, M., Dennison, M., Mabesoone, M.F.J., MacKintosh, F.C., Rowan, A.E. and Kouwer, P.H.J., 2014. "Ultra-responsive soft matter from strain-stiffening hydrogels", Nature Communications, Vol.5, pp. 1-8.

Jiang, M., Lawson, Z.T., Veysel, E., Pervere, S., Nan, T., Robbins, A.B., Feed, A.D. and Moreno, M.R., 2020, "Clamping soft biologic tissues for uniaxial tensile testing: A brief survey of current methods and development of a novel clamping mechanism", Journal of the Mechanical Behavior of Biomedical Materials, Vol. 103, pp. 1-40.

Misra, S., Ramesh, K.T. and Okamura, A.M, 2010, "Modeling of nonlinear elastic tissues for surgical simulation", Comput. Methods Biomech. Biomed. Eng., Vol. 13, pp. 811-818.

Murphy, J.G., 2013, "Transversely isotropic biological, soft tissue must be modelled using both anisotropic invariants", European Journal of Mechanics, A/Solids, Vol. 42, pp. 90–96.

Nunes, L.C.S. and Moreira, D.C., 2013, "Simple shear under large deformation: Experimental and theoretical analyses" European Journal of Mechanics-A/Solids, Vol. 42, pp. 315-322, https://doi.org/10.1016/j.euromechsol.2013.07.002.

Rashid, B., Destrade, M. and Gilchrist, M.D., 2014,"Mechanical Characterization Of Brain Tissue In Tension At Dynamic Strain Rates", Journal Of The Mechanical Behavior Of Biomedical Materials, Vol. 33, pp. 43–54.

Rivlin, R.S., 1948. "Large Elastic Deformations of Isotropic Materials. IV. Further Developments of the General Theory", Phil. Trans. R. Soc. Lond. A., Vol. 241, pp. 379-397.

Schiavi, A., Cuccaro, R. and Troia, A., 2022. "Functional mechanical attributes of natural and synthetic gel-based scaffolds in tissue engineering: strain-stiffening effects on apparent elastic modulus and compressive toughness", Journal of the mechanical behavior of biomedical materials, Vol. 126, pp. 105066.

Sugerman, G.P., Kakaletsis, S., Thakkar, P., Chokshi, A., Parekh, S.H. and Rausch, M.K., 2021, "A whole blood thrombus mimic: constitutive behavior under simple shear", J. Mech. Behav. Biomed. Mater., Vol. 115, pp. 104216.