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EXPERIMENTAL CHARACTERIZATION OF PORCINE KNEE LIGAMENTS

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Abstract. Porcine knee ligaments were experimentally tested employing a professional material testing machine. Both, elastic and viscoelastic characterization were accessed. Four porcine ligaments were utilized in this experimental phase: the lateral collateral ligament (LCL), the anterior cruciate ligament (ACL), the posterior cruciate ligament (PCL) and the medial collateral ligament (MCL). The load versus time results were obtained from the material testing machine outputs. The elastic behavior was determined by analyzing the results at relatively low load target. The viscoelasticity behavior was obtained through the realization of relaxation experimental tests, which consists in a sequence of imposed strains, followed by ligaments relaxation. To full comprehension of the soft tissue's viscoelasticity, the concept of convolution was accessed, through the Fung quasi-linear and Schapery's nonlinear theories. The porcine knee ligaments characterization was done through a curve fitting of the experimental outputs with the utilization of mathematical equations of the quasi-linear viscoelastic theory. The application of the experimental parameters in the quasi-linear equations, showed a reasonable accuracy to Fung's model.

Keywords: knee ligaments, analytic model, viscoelasticity, experimental tests

1. INTRODUCTION

Knee ligaments may be subjected to injuries in sports, overloaded activities or even in regular activities. They are responsible for the stabilization and alignment of the bones and knees. The correct description of its mechanical behavior is fundamental to access the knee ligament's performance.

The knee ligaments mechanical behavior is ruled by a phenomenon called viscoelasticity. It characterizes materials that present both viscous and elastic behaviors. The simplest viscoelastic model is the one that considers that it is a linear phenomenon. This approach assumes that the creep compliance and stress relaxation functions, that rules viscoelastic behavior, depend exclusively on time. Thus, this model is applicable for several materials, like metals. If this model is used to describe the polymers behavior, as presented in (Weinerowska-Bords, 2015), and even to soft tissues behavior, as shown in (Yang and Church, 2006) and (Samur *et al.*, 2005), it produces quite limited results.

Nevertheless, to better characterize biological materials, efforts have been made as in (Fung, 1981), that proposes a quasi-linear viscoelastic theory. This model separates the relaxation function in a linear viscoelastic part and in a nonlinear elastic part. By assuming this simplification, the resolution of the expressions could be made easier, and it was experimentally shown that the resultant curves correspond reasonably well to the real behavior of soft tissues.

Some other remarkable studies have been done for soft biological tissues. In (Woo *et al.*, 1981), it was shown the suitability of application of Fung's model to the canine MCL. (Abramowitch and Woo, 2004) used goat's MCL to improve the obtainment of the quasi-linear viscoelastic theory's constants. Furthermore, (Abramowitch *et al.*, 2004), also, used the quasi-linear model to obtain a more suitable relaxation functions for soft tissues.

In addition, some developments have been proposed to improve the quasi-linear model performance. Some authors presented a so-called adaptive quasi-linear viscoelastic model to accomplish a model to describe the soft tissue's behavior. In (Nekouzadeh *et al.*, 2007) an adequate quasi-linear simplified model was presented, with a non-linear stiffness function and linear integral of Boltzmann called the "viscous strain". The theory was validated by experimental tests with reconstructed collagen. This model was found easier to apply because it was not dependent of a convolution integral. Moreover, (Quaia *et al.*, 2009) tested the original quasi-linear viscoelastic model for the passive eye muscle in primates and it revealed a quite unsatisfactory behavior, but when tested the adaptive quasi-linear model, the performance was found appropriate. Furthermore, (Troyer *et al.*, 2012a) presented a study that has the objective of improving the experimental method to characterize the relaxation function. For this purpose, a spinal anterior longitudinal ligament was tested in relaxation. The improvement requires the development of a finite ramp time, that was made by an algorithm. In addition, (Funk *et al.*, 2000) showed the viscoelastic behavior of ankle ligaments. It was found that quasi-linear model had a good performance until a certain strain value, from which only a nonlinear viscoelastic model could describe accurately the ligament relaxation response.

In fact, many other researchers recognized that biological tissues have, in most cases, a fully nonlinear behavior. Particularly, (Schapery, 1969) presents a generalized single integral nonlinear model, important not only for soft tissues but for a huge number of materials. Accordingly, in a research that proves that both, quasi-linear and nonlinear viscoelastic, theories have an inadequate performance. (Duenwald *et al.*, 2010) shows that for a soft tissue, like the porcine digital tendon, only Schapery's non-linear expression performed well. Similarly, (Provenzano *et al.*, 2001) shows that the separation of time and strain dependence, in quasi-linear viscoelastic theory, was not adequate for all soft tissues.

Moreover, the non-linear viscoelasticity was accessed by various models and for multiple applications, not only in the soft tissue case. Like in (Luo *et al.*, 2007), where the polycarbonate is found to have a nonlinear viscoelastic behavior for most stress values, which behavior corresponds mostly with Findley's simplified multiple integral theory. Similarly, (Pipkin and Rogers, 1968) shows an important generalized model with single integrals that fit with more than one type of material. Yet, in (Pioletti and Rakotomana, 2000) and (Provenzano *et al.*, 2002) presented the nonlinearity of the human knee ligaments' viscoelasticity using continuum theory and convolutional approaches, respectively. Lastly, (Troyer *et al.*, 2012b) used finite element and analytical formulations to characterize the nonlinear viscoelasticity of soft tissues.

The objective of this study is to characterize the viscoelastic behavior of four porcine knee ligaments through doing experimental tests. Double relaxations tests were implemented, obtaining the respective Fung's model experimental constants. Finally, the repeatability/variability of these constants were evaluated.

2. MATHEMATICAL MODELS

The viscoelastic mechanical behavior can be modeled by mathematical theories/models. As it was presented previously, there are many models and variations that were proposed to describe this phenomenon. However, only two of these approaches can be considered really important for soft tissues and knee ligaments applications. They are the quasi-linear and the non-linear models. In both, the convolution approach is used. This approach allows a relatively simple and concise representation of models. However, as it uses nontrivial mathematics, it is important to be detailed.

Convolution can be understood as the summation of the multiplication between two functions, in each point. That means that if two functions are multiplied, in each point, it would generate a different result, and the sum of all these results create another function, different from the original ones. Consequently, it makes sense to say that the convolution only exists when both functions intersect mutually. Besides, viscoelasticity is a concept that involves constitutive equations, in other words, equations that relate stress and strain. For instance, in a simple elastic example, the multiplication of a constant stiffness by the strain, results in the stress. Nevertheless, viscoelastic materials are more complex since stiffness is not a constant and strain and stress depend also on time. Therefore, the constitutive equation cannot be depicted by a simple multiplication as can be done in the elastic model, but by the utilization of convolution approach.

Accordingly, the multiplication of the stiffness function and the strain function results in the stress function, that depends also on time, can be accomplished through the utilization of convolution. This equation is also known as the Boltzmann superposition integral. Thus, the linear viscoelastic single integral convolution equation is shown:

$$\sigma(t) = \int_0^t E(t-s) \frac{d\epsilon}{ds} ds \quad (1)$$

Where $E(t)$ is the stiffness function, that depends on time, and strain enters as a time derivative, result of a chain rule. Furthermore, t is the fixed present time s is the floating time, varying on the integration.

This concept will be applied to almost every viscoelastic model, specially to Fung's and Schapery's models, which can be quite complex. These models have been used in a huge number of viscoelastic studies, showing its importance. Some examples of these studies that utilize the quasi-linear theory, the Schapery's nonlinear theory or both are (Drapaca *et al.*, 2006), (Provenzano *et al.*, 2002), (Duenwald *et al.*, 2009a), (Woo, *et al.*, 1993), (Sarver *et al.*, 2003), (Kwan *et al.*, 1993), (Selyutina *et al.*, 2015), (Dortmans *et al.*, 1984), (Duenwald *et al.*, 2009b). Fung's quasi-linear model and Schapery non-linear model are explained next.

2.1 Fung's Quasi-Linear Viscoelastic Model

In Fung's Quasi-Linear viscoelastic theory, the function that characterizes the behavior of viscoelastic materials with a constant strain, called relaxation function, is divided in two different functions. First, the elastic response corresponds to a non-linear increasing stress due to the rapid increase of strain and it is represented by σ_e . Second, the reduced relaxation function $g(t)$ is a linear function dependent only on time and represents the decreasing stress under the maintenance of an initial strain. Stress equation turns out to be:

$$\sigma(t) = \int_0^t g(t-s) \frac{d\sigma_e}{d\varepsilon} ds \quad (1)$$

The reduced relaxation function occurs since time 0 until infinite or the removal of the strain input. Furthermore, it is normalized by the ratio of stress in present time and the stress in time equal to zero, that is $\sigma(t)/\sigma(0)$, which results in a $g(0) = 1$. This normalization is due to the physical meaning of the function, since 1 is the neutral multiplier, and at the initial point there is no relaxation yet, that means that $g(t)$ should not interfere in the stress function. However, σ_e is uniquely defined for $t = 0$ s. This is due to a hypothesis made for the equation formation, that assumes that strain increases with an infinite rate, like a step. Therefore, the elastic response depends only on strain, since there is no time variation, and enters as a derivative in the integral, producing a chain rule with strain and time.

The mathematical definition of Fung's reduced relaxation function $g(t)$ is based on a continuous relaxation spectrum. This definition includes various functions and even an integral, that can only be solved numerically. Nevertheless, an equivalent empirical function has shown to be more convenient. This equation is based on the relaxation curve's behavior and in the Maxwell generalized model, that combines springs and dashpots in series to simulate the behavior of viscosity and elasticity acting together. This equation, wrote as a Prony series, can be shown as:

$$g(t) = G_\infty + \sum_{i=1}^{\infty} G_i \cdot e^{\frac{-t}{\tau_i}} \quad (2)$$

Where G_∞ and G_i are material constants that have stress units, called relaxation modulus, which the first one represents this number in time infinite. This parameter represents the amplitude of the stress curve in relaxation. Furthermore, τ_i is the relaxation time, also a material constant, modifies the exponential decay of stress with a constant strain according to the viscosity and elasticity modulus. Initially, this Prony series could have many terms, but it was verified, for instance, by (Funk *et al.*, 2000), that no significant gain was obtained using more than three terms.

$$g(t) = G_\infty + G_1 \cdot e^{\frac{-t}{\tau_1}} + G_2 \cdot e^{\frac{-t}{\tau_2}} + G_3 \cdot e^{\frac{-t}{\tau_3}} \quad (3)$$

Lastly, the elastic response can also be represented by an empirical function. This function is based on the stress per strain curve with a step strain (Woo *et al.*, 1981). Its form turns out to be:

$$\sigma_e = A(e^{B\varepsilon} - 1) \quad (4)$$

Where A and B are dimensionless material constants.

2.2 Schapery's Non-linear Viscoelastic Model

The nonlinear viscoelastic theory modeled by Schapery, as it was mentioned previously, is more complex than Fung's one. Its full nonlinearity means that a single function that rules the material's viscoelastic behavior, depends on a much larger number of interrelated variables. However, this complexity enables to generate a quite successful theory, when compared to soft tissues experimental results.

The present model is based on thermodynamical concepts and permits an analysis according not only to the strain or stress level, time, and material parameters, but also with temperature, which is very valuable in various situations. In addition, Schapery's theory, as a non-linear model, allows the characterization of the two fundamental viscoelastic phenomena. Stress relaxation is the first, which was discussed previously. The second is creep, the situation in which the material receives a stress input, that is held constant, and produces a strain modification with time. This last phenomenon is highly nonlinear and due to that, cannot be applied with a quasi-linear model.

Both equations that express the resulting strain and stress are, respectively:

$$\varepsilon(t) = g_0 J_0 \sigma + g_1 \int_0^t \Delta J(\varphi - \varphi') \frac{dg_2 \sigma}{d\tau} d\tau \quad (5)$$

$$\sigma(t) = h_e G_e \varepsilon + h_1 \int_0^t \Delta G(\rho - \rho') \frac{dh_2 \varepsilon}{d\tau} d\tau \quad (6)$$

Where g_0 , g_1 and g_2 are stress dependent coefficients based on thermodynamic concepts. As explained in (Haj-Ali and Muliana, 2003), g_0 is the non-linear elastic response that calculates the instantaneous change in stiffness, g_1 is the non-linear transient response and g_2 is the parameter that measures load rate effects in creep. The letter g and the index numbers indicate the Gibb's free energy dependence and its order. Analogously, h_e , h_1 and h_2 are material functions that depend on strain and Helmholtz free energy. The indexes one and two are related to the order of dependence on the free energy and "e" refers to h in equilibrium.

Moreover, J_0 and ΔJ are respectively the creep compliance components of initial value and transient, where $\Delta J = J(t) - J_0$. These functions, as its name indicates, are the responsible for the material's behavior in creep situations. Equivalently, G_e and ΔG are the relaxation function components where G_e represents the state in equilibrium and ΔG is the transient parameter and $\Delta G = G(t) - G_e$.

These functions depend on time, temperature, and stress, for creep compliance, or strain, for stress relaxation. This multiple dependence is represented by the reduced time functions φ and ρ :

$$\varphi = \int_0^t \frac{dt'}{a_\sigma} \quad (7)$$

$$\rho = \int_0^t \frac{t'}{a_T} \quad (8)$$

Reduced time functions represent the amount of time necessary for a total creep, that means to the stress reach the equilibrium or turn asymptotically constant or achieve a total relaxation. The same situation for strain, respectively, changes with the shift factors. These shift factors are represented in the equations as a_σ and a_T and are material functions that depend on stress or strain, respectively, besides temperature and other parameters. They are based on the time-temperature superposition principle and the time-temperature-stress superposition principle showed in (Pindera, 1981) and (Roth, 2016).

3. EXPERIMENTAL TESTS

Relaxation tests were done with four porcine knee ligaments to characterize this viscoelastic material by the Fung's quasi-linear theory. This model was chosen firstly, instead of Schapery's, because it has a more straightforward application. To use Fung's model there are nine parameters that can be achieved experimentally. Only one relaxation test, per ligament, is necessary to obtain all experimental parameters.

For this research four different porcine knee ligaments were used: the anterior cruciate (ACL), the posterior cruciate (PCL), the medial collateral (MCL) and the lateral collateral (LCL). The experimental method applied was like the one used in (Duenwald *et al.*, 2010), based on three steps. First, it was done a preconditioning, where the ligaments were exposed to ten cycles of 2% strain of 20 s each, Furthermore, 6% strain was imposed at a rate of 0.3 mm/s and held for 100 s. Then, the strain decreased to 3% percent and rested for 100 s, and finally went back to 6% and hold for other 100 s. This load sequence intends to emulate a real knee ligament, including the values.

The load output of a professional material testing machine INSTRON was recorded for each ligament. Fig. 1 show a PCL positioned at the INSTRON testing machine grips.



Figure 1. PCL setted up on INSTRON

The Origin software was used to generate the curves load vs time, shown in Fig. 2.

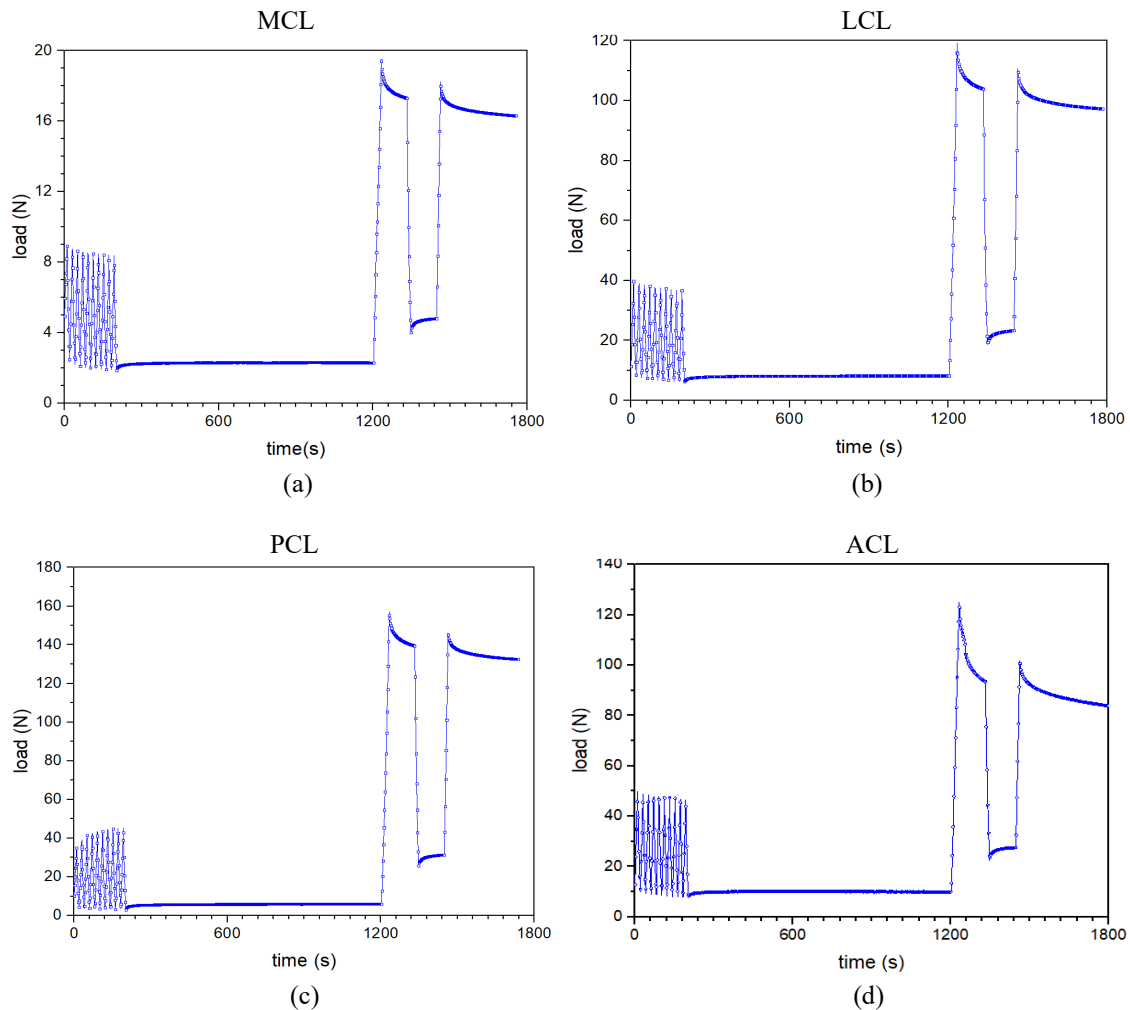


Figure 2. Load vs time graphics of porcine: (a) MCL, (b) LCL, (c) PCL and (d) ACL.

In these graphics it can be observed how load increases rapidly, practically linearly in all four ligaments, followed by two consecutive relaxations. Furthermore, it can be noticed the similarity between the viscoelastic behavior of all knee ligaments. Note, that the second relaxation's stress peak of every ligament is smaller than the first one. Also, the curve turns to an asymptotic behavior more quickly at the second relaxation.

4. DISCUSSION

Using the raw experimental data results, it was possible to post-processing them, to generate stress vs time graphics, necessary to collect the data to generate the input to Fung's model, through the use of the transversal area of each ligament, to obtain the axial stresses. Henceforth, each graphic was divided in two stress relaxation sub-graphics, which were also divided in two curves each. The first curve was the exponential increasing stress that stops in the peak (elastic part), the other starts exactly from the peak and the stress decreases with time (relaxation part).

In Table 1 is shown the ligaments sectional area and ligament length.

Table 1 – Ligaments' Dimensions

	ACL	PCL	LCL	MCL
Sectional Area, mm ²	50.26	95.03	27	20
Length, mm	25.7	27.8	35	32.7

The exponential increasing curves were illustrated in Fig.3.a in a stress per strain graphic and stress relaxation curves are shown in Fig.3.b. The PCL was arbitrarily chosen to generate this example.

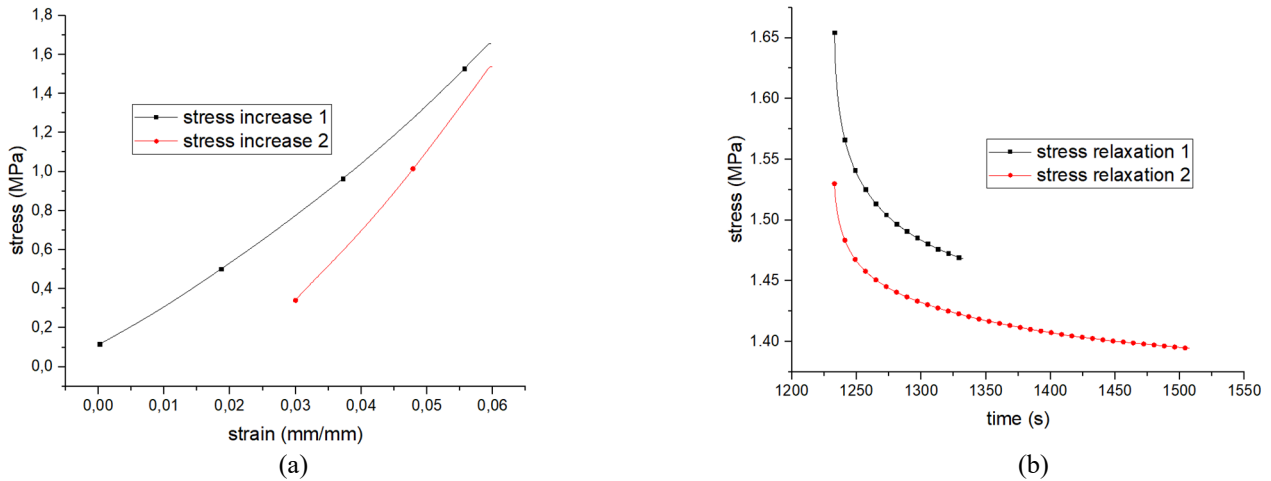


Figure 3. (a) Stress vs strain curves of the PCL of the increasing parts of first and second relaxations and (b) Stress vs time curves of the PCL of the decreasing parts of first and second relaxations

In Fig.3.a, can be observed how stress increases with a nonlinear elastic response of the strain "impulse" or the σ_e term, as explained in the Fung's Quasi-linear Model section. It is also clear that the second curve, represented by the red color, increases faster and in a more linear way. Moreover, its initial strain point is on 3% percent. Differently, the first curve, represented by the black color starts on 0% strain. In Fig. 3.b is shown the ligament's reaction when strain is removed, or the reduced relaxation function $g(t)$. Clearly, the decrease rate is faster on the initial points and gets more slower until it approximates to an asymptotic or equilibrium state. First, when t is near 0 s, the $e^{(-t/\tau_i)}$ terms are near to 1, which results in a high influence of the elastic terms G_i . As time grows, the effect of the viscous terms τ_i also grow, resulting in a larger difficulty to stress decrease. Lastly, it can be observed that the first stress relaxation curve represented by the black color decreases faster than the second, represented by the red color.

Moreover, Fig.3.a shows a stress per strain graphic. These data were used to obtain **A** and **B** constants of equation (4) by curve-fitting accomplished using Origin software. Furthermore, the already set stress per time decreasing curves illustrated in Fig.3.b were applied in the reduced relaxation parameters characterization. Both these estimations by graphic were also used in (Dortmans *et al.*, 1984). Represented in equation (3), G_i and τ_i were found also by curve-fitting, and G_∞ was considered by the software to be the last experimental point. A numerical program can be implemented considering the initial curve and the derivative behavior in order to predict in which stress value the curve will become asymptotic, which is an estimative of G_∞ .

5. RESULTS AND CONCLUSIONS

Using this methodology, the results of all nine parameters, to the double stress relaxations of each ligament, could be found. The parameters were obtained by the first relaxation curve are listed in Table 2, while the constants from the second curve fitting are shown in Table 3.

Table 2 – First stress relaxation results

Material Constants	ACL	PCL	LCL	MCL
G_1 , MPa	0.27	0.03	0.10	0.018
G_2 , MPa	0.18	0.05	0.18	0.032
G_3 , MPa	0.56	0.11	0.32	0.059
G_∞ , MPa	1.81	1.43	3.79	0.86
τ_1 , s	19.33	0.97	1.02	0.59
τ_2 , s	19.6	6.92	7.52	4.73
τ_3 , s	370.8	53.18	53.38	32.29
A , MPa	112.96	1.86	3.94	9.47
B	0.34	10.15	1.19	1.39

In the Table 1, it is presented the parameters, indicated as material constants of the first relaxation curves. As it was said before, G_1 , G_2 and G_3 are the amplitude components of the stress relaxation function $g(t)$. G_∞ is the infinite or equilibrium component, representing the stress with an asymptotic strain. Furthermore, τ_1 , τ_2 and τ_3 are the exponential components of $g(t)$. Lastly, **A** represents the amplitude of σ_e , which means the further stress can go under an increasing strain, while **B** is the exponential component of the same function, dictating the speed of stress growth.

Table 3 – Second stress relaxation results

Material Constants	ACL	PCL	LCL	MCL
G_1 , MPa	0.063	0.028	0.11	0.02
G_2 , MPa	0.098	0.046	0.17	0.03
G_3 , MPa	0.24	0.077	0.24	0.04
G_∞ , MPa	1.64	1.387	3.55	0.81
τ_1 , s	2.35	1.67	1.88	0.96
τ_2 , s	19.86	13.91	15.63	9.38
τ_3 , s	213.64	134.91	150.0	95.36
A , MPa	54.88	1.77	7.27	11.58
B	0.86	12.79	6.17	1.77

The sequential relaxations were input in the same ligaments, but the material constants had different results. This tables show the resulting data of the curve-fitting using Levenberg-Maquardt algorithm. These values had R bigger than 0.99, which demonstrates its precision. Moreover, the standard deviation was not presented since they were in majority smaller than 1% of the parameters value, mostly in the order of 10^{-4} .

With the experimental results, the Fung's quasi-linear viscoelastic expression, for each ligament, could be characterized. The parameters for each relaxation curve were quite similar. For example, the relaxation functions G_i are practically equal for the MCL in Table 1 and Table 2. This is valid for all ligaments, except for the ACL, that are slightly different between the first relaxation and the second one. Furthermore, the values are mostly similar and in the same order of magnitude of the results showed in (Troyer *et al.*, 2012a) and (Abramowitch and Woo, 2004).

Therefore, the similarities of the reduced relaxation functions, for the same ligaments and, in a larger scale, between all four ligaments, demonstrates the precision of the experiment. Furthermore, the predictability of the relaxation times τ_i are also evident, since the values for a same ligament increase with more relaxations, except for the ACL. All these evidences induces that the quasi-linear viscoelastic theory is a simple and quite accurate model for the characterization of the porcine ligament's behavior and its parameters.

Furthermore, the parameters A and B that represent the increasing part of the curves are shown to differ between each ligament. The experimental results obtained are similar to the ones found in (Kwan *et al.*, 1993), that evaluates exactly porcine ACL. Moreover, when comparing the MCL values with the ones shown in (Woo *et al.*, 1981), the values are highly dissimilar. This can be due to the difference between the specimens, since porcine ligaments, obviously, are not identical to canine ones.

Nevertheless, the generalized nonlinear theory modeled by Schapery is considered as a next step for the research of viscoelastic characterization of porcine ligaments. Although the quasi-linear model's efficiency, biological tissues are shown to be fully nonlinear, as it was discussed in the introduction, and it was demonstrated by (Provenzano *et al.*, 2002) that for soft tissues application, Schapery's model must be used.

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