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COB-2021-1114 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. 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 achieve a better comprehension of the soft tissue's viscoelasticity, the concept of convolution was accessed and implemented in 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 satisfactory performance of Fung's model.*

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

1. INTRODUCTION

Knee ligaments are one of the most injured parts of the body in function of sport activities, age or impact actions. They are responsible for the stabilization and alignment of 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 approach 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 simpler, and it was experimentally shown that the resultant curves correspond reasonably well to the real behavior of soft tissues.

Some other notable studies have been done employing soft biological tissues. In (Woo *et al.*, 1981), it was shown a 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 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 had the objective of improving the experimental method to characterize the relaxation function. For this purpose, a spinal anterior longitudinal ligament was submitted to a relaxation test. The improvement requires the development of a finite ramp time, that was implemented by the utilization of a dedicated 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 vast number of materials. (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 for 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 adequately 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 the analysis of the experimental tests results. 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 of a material can be modeled by mathematical theories. As it was introduced previously, there are many models and variations that had already been proposed to describe this phenomenon. However, only two of these approaches can be considered especially significant for soft tissues and knee ligaments applications. They are the quasi-linear and the non-linear models. In both, the convolution method 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 of a sequence of points. That means that if two functions are multiplied, in each point, the sum of all these results would 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, the viscoelasticity 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 correspondent 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 application of convolutional approach.

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

$$\sigma(t) = \int_0^t E(t-s)\frac{d\varepsilon}{d\tau}ds \tag{1}$$

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 is 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 several viscoelastic studies, showing its importance. Some examples of these studies that utilize the quasi-linear theory, the Schapery's nonlinear theory or both are (Selyutina *et al.*, 2015), (Duenwald *et al.*, 2009b), (Duenwald *et al.*, 2009a), (Drapaca *et al.*, 2006), (Sarver *et al.*, 2003), (Provenzano *et al.*, 2002), (Woo, *et al.*, 1993), (Kwan *et al.*, 1993), (Dortmans *et al.*, 1984). 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:

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

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 σ(t)/σ(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 development, 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:

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

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. Thus, the equation (2) can be rewritten to:

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

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

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

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 approach, when compared to soft tissues experimental results.

The present model is based on thermodynamical concepts and permits the analysis according not only to the strain or stress level, time, and material parameters, but also in function of temperature, which is 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 one is creep, the situation in which the material receives a stress input, that is held constant, and produces a strain changing along the time. This last phenomenon is highly nonlinear and due to that, cannot be effectively described by a quasi-linear model.

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

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

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

Where g_o, 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 Helmoltz 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_o 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:

$$\varphi = \int_o^t \frac{dt'}{a_\sigma} \tag{7}$$

$$\rho = \int_0^t \frac{t'}{a_T} \tag{8}$$

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. 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 made with four porcine knee ligaments to characterize its viscoelastic behavior, by the application of 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 accomplished 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, Additionally, 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 comportment.

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 versus time, shown in Fig. 2.

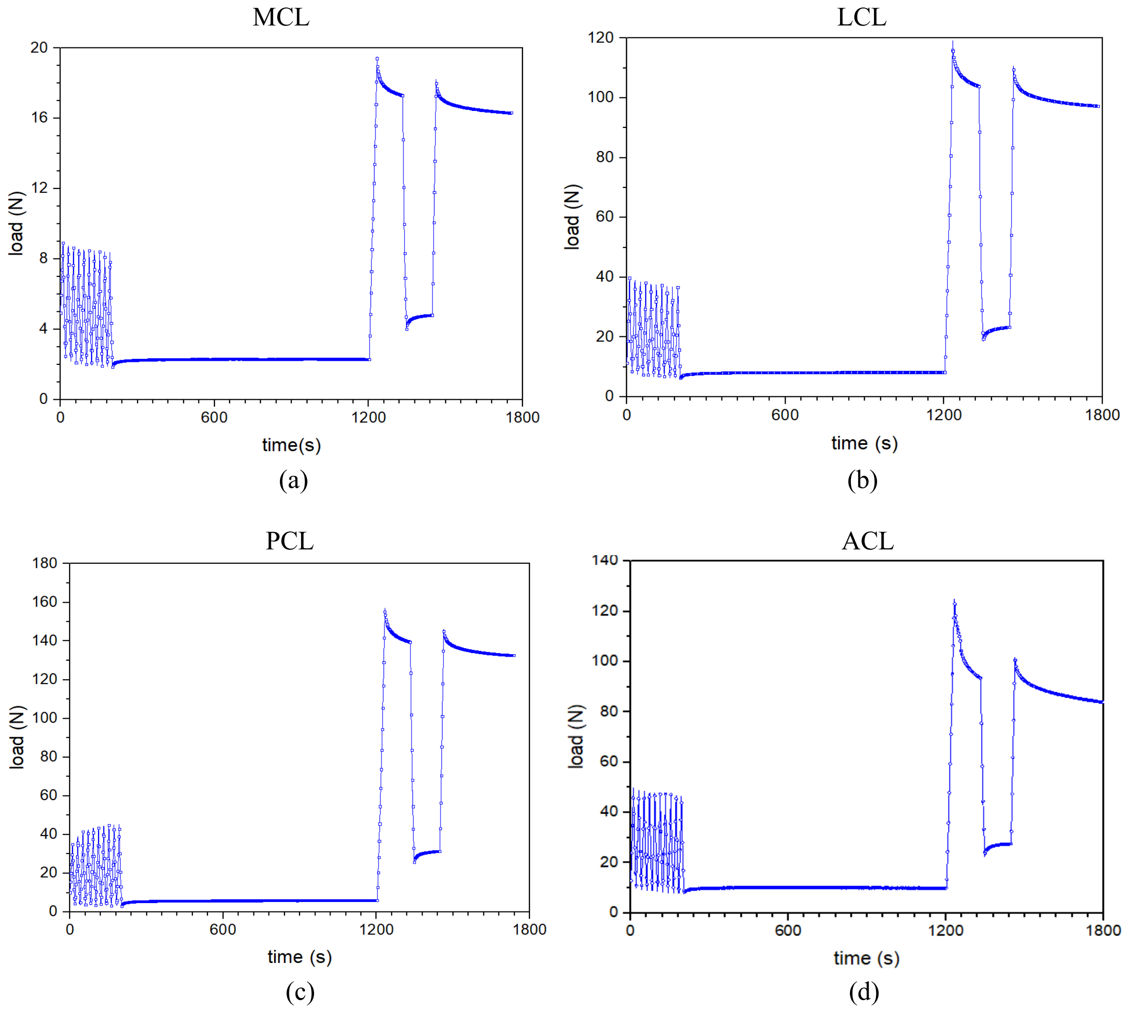


Figure 2. Load versus time graphics for porcine ligaments: (a) MCL, (b) LCL, (c) PCL and (d) ACL.

In these graphics it can be observed how load increases rapidly, practically linearly in all four ligaments, followed by two consecutives 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, was smaller than the first one. Also, the curve tends to an asymptotic tendency more rapidly at the second relaxation.

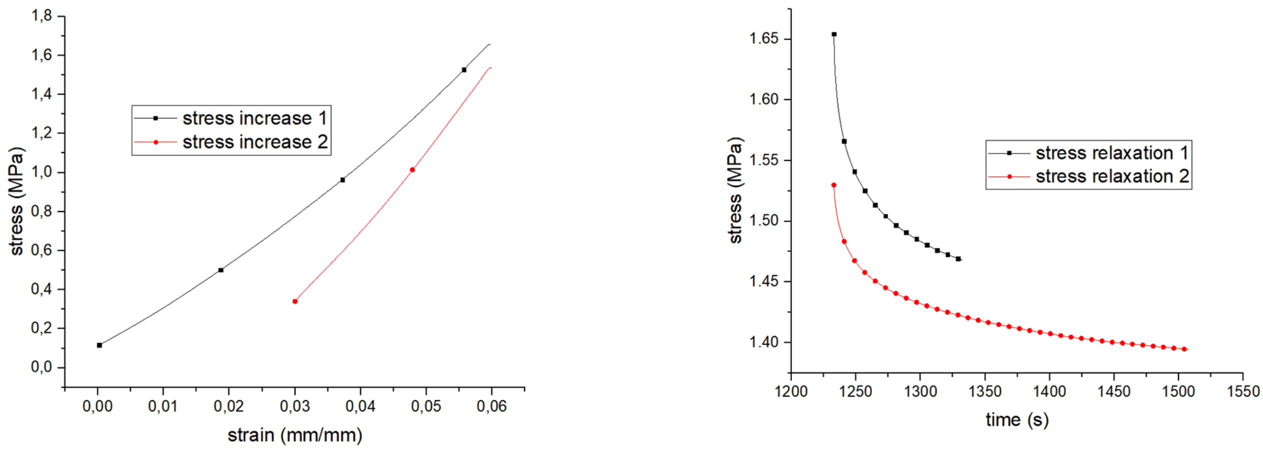
4. DISCUSSION

The experimental results have been post-processed, using the transversal area of each ligament, to generate stress versus time graphics. It was necessary to generate the input data to Fung's model. Henceforth, each graphic was divided in two stress relaxation sub-graphics, which were also divided in two curves each. The first curve was described by the exponential increasing stress up to the peak (elastic part) and the other curve starts at the peak, with the stress decreasing along the time (relaxation part). In Table 1 it is shown the ligaments cross sectional area and 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 versus strain graphic and the stress relaxation curves are shown in Fig.3.b, in a stress versus time graphic. The PCL was, arbitrarily, chosen to generate this example.



(a) (b)

Figure 3. PCL porcine ligament: (a) Stress versus strain curves and (b) Stress versus time curves.

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 than the first one. Note that the first curve, represented by the black color starts on 0% strain and the second curve, represented by the red color its initial strain point is on 3% percent. In Fig. 3.b are shown the ligament's reduced relaxation functions 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 pass, the effect of the viscous terms τ_i also grows, 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.

The Fig.3.a shows a stress versus strain graphic, which was used to obtain *A* and *B* constants of equation (4) by curve-fitting, using Origin software. The stress versus time decreasing curves illustrated in Fig.3.b were applied in the reduced relaxation parameters characterization. Both these graphical estimations 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 to predict in which stress value the curve will become asymptotic, estimating G_∞.

5. RESULTS AND CONCLUSIONS

Using this methodology, the results of all nine Fung's 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 relaxation curve 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	0.02
G_2, MPa	0.18	0.05	0.18	0.03
G_3, MPa	0.56	0.11	0.32	0.06
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, presents the parameters, indicated as Fung's 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 at 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 and *B* is the exponential component of the same function, determining the speed of stress growth.

The second stress relaxation results of the same ligaments revealed differences in Fung's material constants.

Table 3 – Second stress relaxation results

Material Constants	ACL	PCL	LCL	MCL
G_1, MPa	0.06	0.03	0.11	0.02
G_2, MPa	0.10	0.05	0.17	0.03
G_3, MPa	0.24	0.08	0.24	0.04
G_∞, MPa	1.64	1.39	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

This tables show the resulting data of the curve-fitting using Levenberg-Maquardt algorithm. These values had *R* bigger than 0.99, which reveals its accuracy. 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).

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 adequate model for the characterization of the viscoelastic porcine ligament's behavior. 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 like the ones found in (Kwan *et al.*, 1993), that evaluates just the porcine ACL.

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