EXPERIMENTAL STRESS-STRAIN CURVES FOR THE KNEE CRUCIATE LIGAMENTS

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Abstract. *Many of the injuries experienced by human and other animal knees are associated with the anterior cruciate ligament (ACL). The mechanical characterizaation of knee ligament properties is an important step towards the development of rehabilitation techniques and biomechanical systems. This work presents an experimental study performed for the mechanical characterization of soft tissues comprised of collagen fibers. The experimental apparatus used in this work includes a universal testing machine, in which the axial load and elongation can be monitored during the tests, and a special gripping system designed for ACL specimens. Uniaxial tensile tests are performed on nine specimens of canine knee cruciate ligaments at three deformation speeds – 1 mm/s, 4 mm/s and 8 mm/s. All tissues are removed from the knee cavities, except the anterior cruciate ligament. The experimental data is employed to build the stress-strain curves of canine ACL specimens and to estimate some of their relevant mechanical properties, such as the ultimate axial stress and the tangent modulus.*

Keywords*: Knee Kinematics, Cruciate Ligaments, Soft Tissues, Biomechanics.*

1. INTRODUCTION

Knee injuries are a problem that affects two people out of each group of thousand people worldwide. In 90% of the knee injuries, the cause is located either on the anterior cruciate ligament (ACL) or on the medial collateral ligament (MCL) (Miyasaka *et al.*, 1991). Ligament lesions can produce different levels of motion instability and high rates of collateral injuries (Mello *et al.*, 1999).

The determination of the knee cruciate ligament mechanical properties is crucial for a better understanding of the knee Kinematics, of the mechanisms associated with the ligament lesions, and of the several treatment techniques. The most common procedure employed to evaluate the ligament mechanical properties is the uniaxial tensile tests, which render the curves of stress-strain for the ligaments (Fung, 1993).

The soft tissues comprised of collagen fibers, such as the ligaments, present a viscoelastic behavior, which is characterized by the combination of behaviors of elastic and viscous materials (Fung, 1993). Experimental testing conditions, such as the gripping technique and the test speed, affect strongly the stress-strain curves for soft tissues, which can result in large discrepancies among experimental curves presented by different authors (Wang *et al*., 1997; Pioletti, 1997; Azangue *et al.*, 2000; Resende *et al.*, 2005).

This work presents an experimental study carried out to characterize some mechanical properties of canine knee ligaments through uniaxial tensile tests. This work also aims at discussing some methodological aspects involved on the mechanical characterization of soft tissues. The experimental setup employed in this study includes a universal materials testing machine, which permits the monitoring of the axial load and displacement during the tests, and a special gripper specially designed for this work. Curves of stress-strain are obtained from the uniaxial tensile tests performed on nine specimens of canine ACL at three deformation rates: 1 mm/s, 4 mm/s and 8 mm/s. The results rendered in this study show that the ligament axial strength and the tangent modulus increase as the deformation rate increases.

2. EXPERIMENTAL SETUP AND PROCEDURES

Nine specimens of canine ACL ligaments are selected for this investigation. In the specimen preparation, all tissues except the ACL are removed from the knee joint. The specimen set is collected from adult dogs of undefined dog Breed with weight varying from 34 kg to 36 kg and with age from 2 to 6 years, at the Veterinary School of UFMG. The specimens include the femoral and tibial bone ends and the ACL. The specimens are conditioned in saline solution and then frozen at -15º C. Twelve hours before the tensile tests, they are brought to ambient temperature. The mechanical tests performed on the ACL specimens are carried out at ambient temperature. During all stages of conditioning and preparation for the tests, the specimens are moistened by using a saline solution of 0.9%. All experimental procedures employed in this work have been approved by the Ethics Committee for Animal Experiments (CETEA/UFMG).

Nine stress-strain curves are generated for the nine ACL specimens evaluated. Three specimens are grouped to be tested at each deformation rate selected. Three values of axial stress computed for each test group are averaged to permit the determination of the average stress-strain curve at each deformation rate. In all tests it has been observed the ACL rupture.

Figure 1 depicts the Instron universal materials testing machine used in this work. This an electromechanical machine, Instron model 5869, controlled by computer and with a force transducer of 5 kN. This machine belongs to the physical tests laboratory of the Technological Center of Minas Gerais Foundation (CETEC-MG). The testing system uses acquisition electronics and the standard industrial software Bluehill®. This experimental apparatus provides automatically the values of axial strain and load by using extensometers and load cells.

Figure 1. Universal materials testing Instron machine, model 5869, from the physical tests laboratory at CETEC-MG.

For the computation of strain and stress on the ACL specimens, some measurements of the ACL length and cross-section characteristic diametral dimensions are needed. With the ACL specimens mounted on the testing machine, an axial force of 2 N is applied and, then, an analogical caliper with resolution of 0.01 mm is employed to measure the ACL geometric parameters of interest. The ACL length is measured since the tibial insertion until the femoral insertion. Three measurements of each specimen length and characteristic diameters are taken for estimating the average specimen length and diameters used for stress computation. The ACL average transverse cross-section area is computed by assuming that this cross-section area has an elliptical form. Noteworthy to say that the measurement device employed to evaluate the ACL geometric properties can be a strong source of inaccuracies. Handling soft tissues with measuring contact devices, such as calipers and micrometers, is a methodological drawback of many works developed to characterize the mechanical properties of ligaments (Pioletti, 1997; Sekiguchi *et al*., 1998; Panjabi and Courtney, 2001).

A special gripping system has been devised to attach the ACL specimens to the testing machine. Figure 2 depicts a picture of the side view of this gripper, which has been developed jointly by CDTN (Center for Development of Nuclear Technology) and UFMG (Resende *et al.*, 2005). This gripper is comprised of metallic tubes and plates, which are connected by using screws. Special holes are introduced into the tubes for the screws used to hold firmly the specimen bone ends. The lower part of this gripper is rigidly mounted and its upper part has some relative motion that permits adjustments to accommodate specimens of different lengths and transverse areas (see Fig. 2). The femoral bone end is attached to the upper part of the gripper, which allows adjusting the angle between the centerlines of the bone ends. The tibial bone end is attached to the rigid part of the gripper. During the mounting of the specimens on the gripper, a great effort is made to guarantee the parallel alignment of the ACL collagen fibers. In order to reduce the looseness between the gripper metallic parts and the bone ends, these bone ends are inserted into PVC commercial tubes and industrial adhesives are employed to diminish any relative motion between the gripper and the specimen. The methodological approach based on uniaxial tensile tests permits only to evaluate the ACL specimens as single structures, even though these specimens comprise of two bundles, which are the anteromedial and the posterolateral ones.

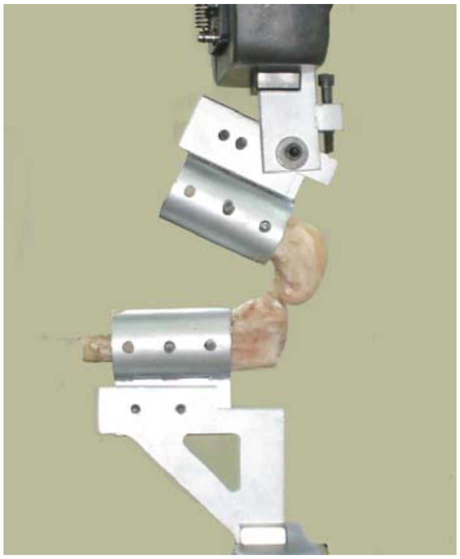

Figure 2. Picture of the gripping system specially devised to test ACL specimens with bone ends.

(a) ACL specimen before the tests. (b) ACL deformation during the tensile tests.

Figure 3. Pictures of the ACL specimens during the tensile tests.

Figure 3 shows two moments of the ACL specimen during the tensile test – immediately before the axial load is started and when the specimen axial elongation reaches a large value. The nine ACL specimens are randomly divided into three test groups. Each group is designed to be tested at a pre-selected deformation rate. The deformation rates chosen for this investigation are 1 mm/s, 4 mm/s e 8 mm/s.

3. EXPERIMENTAL RESULTS

The results rendered by the uniaxial tensile tests are employed to generate the stress-strain curves and to obtain some mechanical properties for the ligament, such as ultimate axial stress, ultimate axial strain and tangent modulus. Furthermore, the experimental data resulting form this study permit to analyze the influence of the deformation rate on the mechanical characterization of viscoelastic soft tissues. The expected time-dependent behavior of the stress-strain curves for the ACL specimens is assessed by performing the tensile tests at different deformation rates.

Figure 4 depicts the average stress-strain curves obtained at three values of deformation rate of 1 mm/s, 4 mm/s and 8 mm/s. The stress-strain curve patterns show clearly the non-linear behavior of the ACL and provide some insights about the strong influence of the deformation rate on the stress-strain relationship. Similarly to other studies presented in the technical literature of Biomechanics (Woo *et al*., 1986; Pioletti *et al*., 1999; Moon *et al*., 2005), the results indicate that as the deformation rate increases the stress-strain curve slope becomes more accentuated, as shown on Fig. 4. The curves depict time-dependent behavior, which is a typical behavior of viscoelastic materials (Crandall *et al.*, 1978). This increasing trend of the curve slope as the test speed increases show that the ACL stiffness to axial elongation increases as the axial deformation rate increases. That means that the axial strength of the ACL becomes larger for higher axial elongation rates.

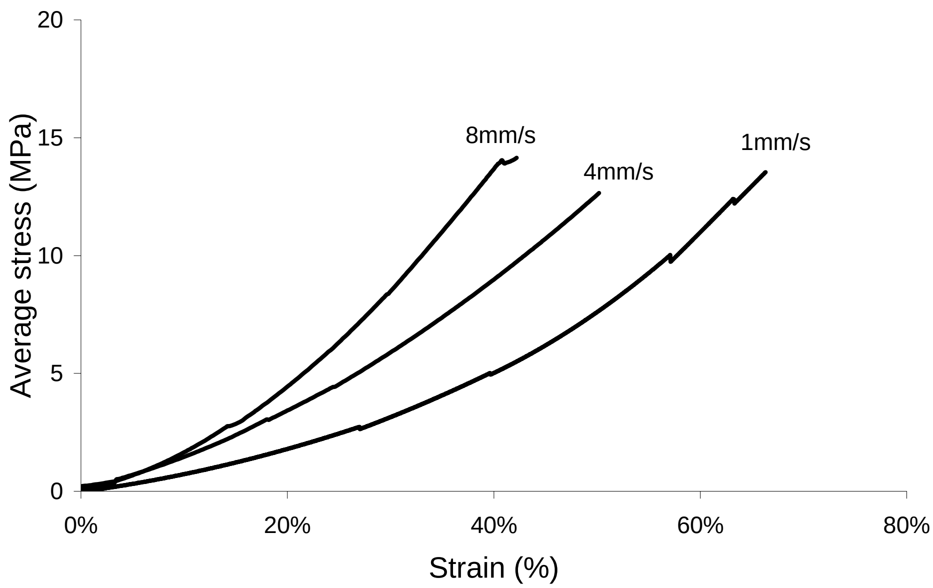

Figure 4. Average stress-strain curves at three deformation rates.

Assuming that the transverse cross-section of the ACL is approximately elliptic, the values of the axial ultimate stress are also estimated from the experimental results obtained through the tensile tests. Table 1 presents the estimates for the axial ultimate stresses for the ACL specimens at three deformation rates. Even though the axial stiffness increases as the deformation rate increases, indicating an increasing trend for the ACL axial strength (see Fig. 4), Table 1 shows that the ultimate stress decreases as the deformation rate increases.

Table 1 . Average values of axial ultimate stress at three deformation rates.

Deformation rate	**Average ultimate stress (MPa)**
1mm/s	23.05
4mm/s	21.61
8mm/s	18.65

For non-linear materials, such as the ACL specimens, the tangent modulus is used to characterize the slope of the stress-strain curves at each level of strain. In this work, the tangent modulus computation is simply performed by dividing the axial stress by the axial strain. The values of tangent modulus serve exclusively as an indicator of the axial

ACL stiffness, since there is a large variation on the computed values. Table 2 present several estimates for the tangent modulus computed by using the average strees-strain curves at three deformation rates. These data depict the increasing trend of the ACL axial stiffness as the deformation rate increases.

Table 2. Tangent modulus evaluated at several levels of axial strain for three deformation rates.

Strain	**Tangent modulus (MPa)** **1 mm/s**	**Tangent modulus (MPa)** **4 mm/s**	**Tangent modulus (MPa)** **8 mm/s**
0.2%	9.59	52.82	110.87
0.4%	6.89	29.90	57.92
0.8%	3.68	18.47	31.61
3%	5.71	10.29	13.46
6%	6.50	14.02	14.03
8%	6.90	14.24	15.24
10%	7.27	14.62	16.55
15%	8.14	15.90	19.07
20%	8.97	17.12	22.19
25%	9.80	18.14	25.28
30%	10.48	19.64	28.27
40%	12.54	22.46	34.18

4. DISCUSSION

As expected, from the stress-strain curves rendered in this work, the deformation rate of the uniaxial tensile test plays a very important role on the mechanical characterization of living tissues, since it strongly influences the stress-strain curve shape and other mechanical parameters. Experimental mechanical characteristics of soft living tissues, mainly those comprised of collagen fibers, must be cautiously analyzed because of their strong dependence on the deformation rate and on several testing parameters and conditions. A clear definition of the appropriate deformation rate for the uniaxial tensile test has to be initially made based on the desired use of the experimental data. For example, in the studies about the ligament rupture, the uniaxial tensile tests should be performed at speeds similar to those experienced by the ligaments during the actual events (Brendolan, 2000). The comparison among experimental results for ligament mechanical properties presented in the technical literature is troublesome due to the large variation of deformation rates employed to perform the mechanical tests.

A very important aspect to be considered in the analysis is the particular characteristic behavior of each specimen. Even on a group of animals from the same species and similar physical characteristics, large variations on the constitutive and geometric parameters are usually encountered. Large samples could improve the reliability of the experimental results from a statistical standpoint. However, technical and ethical difficulties are commonly found on the collection of specimens.

For the computation of the axial stress and strain, measurements of some geometric characteristics, as the specimen length and transverse cross-section diametral dimensions, must be carried out. In the technical literature, these measurements are based on different devices and methodologies. The most common device employed to perform the measurements of soft tissue geometric characteristics has been the analogical caliper. Since calipers require mechanical contact with the specimen, it is usual to observe expressive variations on the dimensions of soft tissues. Another technical difficulty consists on the measurement system accuracy. ACL specimens generally have small dimensions and any measurement small variation can lead to large variations on the computation of the mechanical properties.

There is a controversy in the literature of Biomechanics with respect to the relationship between the ultimate stress and the deformation rate. In this study, the values of the axial ultimate stress decrease as the deformation rate increases. Oppositely, the work of Kennedy *et al*. (1997) presents experimental results obtained through tensile tests showing that the ultimate stress increases as the deformation rate increases. In their work, the maximum axial load is reached at the highest deformation rate. On the other hand, the study performed by Wang *et al*. (1997) concludes that the ultimate stress does not depict sensitivity to variations of the deformation rate. According to Wang *et al*. (1997), soft tissues, as those that constitute the ACL, arteries and veins, exhibit mechanical properties much less sensitive to variations on the deformation rate than those related to other kind of living tissues, as those that form the periodontal ligament, the heart passive tissue and other longitudinal ligaments.

The experimental results obtained in this work show that the values of the axial ultimate stress vary expressively even for a specified deformation rate. The sample sizes are too small and the standard deviation is too large for each group of three specimens tested. The standard deviations for each group of three values of ultimate stress are equal to 5.6 MPa, 4.2 MPa and 3.2 MPa at, respectively, the deformation rates of 1 mm/s, 4 mm/s and 8 mm/s. This only confirms that biological tissues have particular properties that have been defined along the individual way of life. The large variation of experimental results for soft tissue mechanical properties makes very difficult to perform any type of comparison among experimental results obtained from different works. Specimen standardization for soft living tissues is still a matter that deserves to be properly dealt by the scientific community in Biomechanics.

The pattern depicted by the values of tangent modulus, given on Tab. 2, is compatible with the trends shown on the stress-strain curves. A deformation rate increase corresponds to a raise on the ligament axial stiffness. Stiffer ligaments offer more resistance to the ACL axial elongation, raising the ligament strength to axial motion. However, the axial ultimate stress presents a decreasing trend as the deformation rate increases, showing that the ACL will fail at lower axial loads when the tensile tests are performed at higher speeds. Moreover, the ultimate axial strain will be smaller at higher deformation rates.

5. CONCLUSIONS

This experimental investigation shows that the behavior of soft tissues composed of collagen fibers is strongly affected by the deformation rate used in the tensile tests performed for their mechanical characterization. The time-dependent stress-strain relationship is an important feature of the knee cruciate ligaments and must be considered on the knee Kinematics analysis and on the development of new surgical and rehabilitation techniques for the ligaments. The higher is the deformation rate applied on the cruciate ligament, the higher is its axial stiffness. On the other hand, the experimental results obtained in this work show that the maximum axial load sustained by the ACL diminishes as the elongation rate increases.

Methodological aspects and parameters associated with the tensile tests of soft tissues must be evaluated carefully for the process of the tissue mechanical characterization. One of the major technical difficulties is associated with the measurement system used to take the specimen dimensions of interest. Calipers and micrometers, usual measurement devices for small dimensions, exert some level of contact pressure on the soft tissues, which are not even able to sustain small contact forces. Naturally, some dimensional variations occur during the measurement of the specimen geometric parameters using contacting devices, which can affect expressively the computation of the axial stress and strain. Furthermore, the gripping technique for soft tissues consists on another great technical difficulty. It is highly recommended to prepare the ACL specimens with their femoral and tibial bone ends in order to permit their efficient attachment to the testing machine, without generating any change on the ligament fibers.

6. ACKNOWLEDGEMENTS

The financial support provided by FAPEMIG (Process TEC 256/03) is gratefully acknowledged by the authors.

The authors also wish to express their sincere thanks to Professor Rafael Resende Faleiros, from the Department of Veterinarian Clinics and Surgery, of the Veterinary School of UFMG, for his crucial assistance and support in the preparation of the specimens used in this work.

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