Mechanical Properties Characterization of Knee Cruciate Ligaments Through Tensile Tests

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***Abstract**. The mechanical analysis of knee ligaments have brought important contributions to the development of novel rehabilitation therapies, surgical procedures and biomechanical equipments. The cruciate anterior and posterior ligaments, which consist basically on collagen longitudinal fibers, play an important role on the knee joint motion and stability. These ligaments present fundamentally a viscoelastic behavior and the determination of their mechanical properties is a vital step for understanding their influence on the knee joint kinematics. The technical literature lacks not only a well founded experimental methodology for testing soft tissues but also does not bring significant experimental data about the constitutive behavior of cruciate ligaments. This paper describes an experimental study carried out to determine some mechanical properties of canine cruciate ligaments through tensile test. Technical aspects related to the testing apparatus andto to the methodological difficulties associated with soft tissue tensile tests are described in conjunction with some experimental curves for load versus displacement and stress versus deformation rate, which are obtained from testing some specimens. The results show clearly the non-linear constitutive behavior of the ligament tissue and demonstrate the importance of selecting appropriate loading time rates in order to identify correctly the peculiar patterns of the ligament properties.*

***Key-words**: Cruciate ligaments, Knee joint, Viscoelastic material, biomechanics*

1. Introduction

Knee ligament injuries are a health problem that affects seriously not only human beings but also several animals, such as horses, dogs, cats, rabbits and others. About ninety percent of all knee injuries are related to the anterior cruciate ligament (ACL) and to the medial collateral ligament (MCL) (Miyasaka *et al.*, 1991). In order to enlarge the knowledge about the role played by the ligament structure on the knee motion, many scientific studies have been promoted to develop new technical procedures and to improve the classical methodologies currently employed to analyze the knee ligaments.

The determination of the ligament mechanical properties is a fundamental step to understand the influence of the ligaments on the knee kinematics, to evaluate the mechanisms associated with the knee injuries and to develop treatment techniques. Many of the ligament mechanical properties can be obtained by performing uniaxial tensile tests on ligament specimens. Many works reported on the technical literature about knee ligament properties describe tensile tests performed on soft tissues (Pioletti, 1997), however there is not a unique neither a well-founded experimental methodology for those tests. The ligaments are composed of collagen fibers that behave basically as a viscoelastic material. Consequently, experimental setup parameters used during the tensile tests, such as loading time rate and pre-conditioning, can cause expressive variations on the characteristic curves patterns for ligament tissues.

Pioletti *et al.* (1998) discuss the constitutive behavior of soft tissues analyzing the high sensitivity of the stress-strain curves to the deformation rate. They perform uniaxial tensile tests on specimens of ACL, PCL (posterior cruciate ligament) and patellar tendon, at four different elongation time rates, which are 0.3 mm/s, 6 mm/s, 9 mm/s and 12 mm/s. The specimens are prepared with their bone insertions and undergo a pre-conditioning at elongation rate of 0.3 mm/s. Then, the tensile tests are carried out after elapsing 30 minutes from the pre-conditioning.

Panjabi *et al.* (2001) perform several tensile tests on the ACL of rabbits to analyze the effects of the subfailure stretch on the ligament properties. The ligament specimens include the tibial and femoral bone ends to allow their mounting on the experimental apparatus. An elongation rate of 1.01 m/s has been used in these tests. All specimens have undergone pre-conditioning, which has been carried out for 10 loading cycles at frequency of 1 Hz. Nakano *et al.* (2000) evaluate biomechanically the interference screw fixation of the doubled flexor tendon graft in the reconstruction of the ACL. They compare their technique with some standard fixation procedures and employ a specimen pre-conditioning with 10 elongation cycles at elongation rate of 0.83 mm/s. The specimens consist of femur-graft-tibia sets and are tested through uniaxial tensile tests.

The technical biomechanical literature presents different techniques to fix the specimen samples to the testing machine. Yamamoto *et al.* (2000) employ acrylic blocks attached to both bone ends with collagen fascicles from rabbit patellar tendons in order to carry out the tensile tests. They use cyanoacrilate adhesives for specimen gripping and a special microtensile testing machine in their study. Brendolan (2000) devises a fixation system based on PVC commercial pipe with lateral holes to screw the tibial and femoral bone ends. In her work, the bone ends are inserted into the PVC pipes filled with acrylic resin in order to prepare the canine fascia lata and ACL specimens for the uniaxial tensile tests. Then, a special gripper is specially developed to receive the PVC pipe-bone ends sets.

This work presents the preliminary stages of an experimental study about the mechanical characterization of canine ACL specimens performed through tensile tests. Two different specimen gripping techniques are used in conjunction with two elongation time rates in order to discuss some methodological aspects involved in the experimental analysis of collagen fibers of soft tissues. The experimental setup employs a universal tensile testing machine with mechanical transducers to measure the axial load and displacement. Curves of load versus displacement and stress versus deformation rate obtained from six specimens are shown in this work. Some technical difficulties associated with the gripping techniques used in this work bring important subsides for designing a novel gripper for testing knee cruciate ligaments and other collagen structures.

2. Methodology and experimental description of the tensile tests

An Instron testing machine (Instron, Ltd, Canton, Massachusetts), which is installed on the Mechanical Testing Laboratory of the Center for Development of Nuclear Technology (CDTN), has been used to perform the tensile tests on the canine ACL specimens in this work. The mechanical testing system includes a load cell of 500 kgf, which has been calibrated for a range from 0 to 100 kgf, a displacement transducer, which allows to measure displacements in the range from 0 to 50 mm, and a data acquisition system Agilent, model 34970 A. The tests are performed at ambient temperature. A more detailed description of the methodology is presented as follows.

2.1. Specimen Preparation

Six fresh knee joints of young dogs, at the average age of 4.5 years, have been prepared by the Pathology unit from Veterinary School at the Federal University of Minas Gerais. Four canine ACL specimens were prepared with the tibial and femoral bone ends. Two ACL specimens were completely removed from their tibial and femoral insertions. The surrounding soft tissues were carefully removed from all the specimens.

All specimens were frozen at temperatures about -15º C. The specimens have been removed from their freezing conditions and taken to ambient conditions 12 hours before testing. During their preparation and mechanical tests, they were kept in saline solution of 0.9 %. The tensile tests were performed at ambient temperature. The average specimen length has been estimated from three measurements performed using a caliper with resolution of 0.01 mm. The ACL length was measured from the insertion point at the femoral plateau until the insertion point at the tibial end three times. An average ACL length was employed in the strain computation. The cross-section transverse area of the ACL specimens has been estimated assuming that this cross-section is approximately circular. Three measurements of the anterior-posterior and medial-lateral diameters were carried to estimate the average ligament diameter for stress computation.

2.2. Specimen Gripping

Two different gripping techniques are employed to perform the ACL tensile tests. Firstly, a special gripper built with two small metallic plates is used to attach the specimens to the testing machine. Figure 1 shows the tensile tests in which the specimens are directly attached to the gripper. Two screws in conjunction with sandpaper are employed to fix the collagen fibers to the gripper. This technique must be used very carefully to avoid experimental drawbacks. It is a hard task to insert the specimen in between the plates keeping the effective attachment section constant. Not only variations of the specimen length but also collagen fiber misalignments occur too often when this gripping system is used. A major problem observed during the tensile tests with this gripper has been the sliding between the specimen and the metallic plates.

(a) before load application (b) after load application

Figure 1. ACL specimen attached directly to a gripper built with two metallic plates.

To avoid the experimental problems that happen when the gripper of Fig. 1 is employed, a second gripping technique has been used in this work. The ACL specimens prepared with the femoral and tibial bone ends are attached directly to the testing machine using two long ½ inch screws. Figure 2 depicts the preparation of the specimens using a special gripper built with a screw welded to a small metallic plate for attachment to the testing machine. A manual drilling machine is used to drill the holes on the bone ends for the screws. A major experimental difficulty consists on keeping the angle between the centerlines of the bone ends at approximately 120° to avoid misalignments on the collagen fibers. The methodological approach based on uni-axial 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. A special surgical adhesive is employed to strengthen the bonds between screw and bone.

Figure 2. Gripper built with screws welded to small metallic plates.

Figure 3. Gripper attached to the testing machine.

2.3. Elongation time rate

The experimental apparatus allows varying the specimen elongation time rate from 0 to 0.83 mm/s. The elongation time rate is a test parameter that plays a very important role on the collagen fiber mechanical response. Viscoelastic materials are very sensitive to the load application speed and, consequently, their stress values are usually plotted versus strain time rate. In the biomechanical literature, many authors use the stress-strain curve or the stress-strain rate curve to characterize the mechanical properties of ligaments (Pioletti, 1997; Yamamoto *et al.*, 2000).

In the tensile tests performed in this work, the maximum axial load was set at 50 kgf and the tests were carried out at four different elongation time rates, which were 0.017 mm/s, 0.17 mm/s, 0.3 mm/s and 0.83 mm/s. Experimental data related to time, displacement and axial load have been automatically stored by the data acquisition system. Measurements of the specimen length and diameter were performed with manual instrument.

3. Experimental data

In the first part of this work, the two ACL specimens without the bone ends are placed in the testing machine. The technical difficulties associated with the gripping technique shown in Fig. 1 permit to carry out the tensile tests on these specimens only at elongation time rate of 0.17 mm/s. Figures 4 and 5 depict the curves of axial load versus displacement and stress versus strain time rate, respectively, obtained by the tensile tests performed on the ACL specimens attached directly to the gripper. The oscillations appearing on these curves are due to the sliding between the ligament and the two metallic plates. Also, those oscillations are likely caused by the early rupture of some collagen fibers, which ends are strongly compressed between the fixing plates. The mechanical strength of the collagen fibers close to their ends are strongly reduced when the plates are pressed one to another. Despite the experimental drawbacks on this first section of the tensile tests, the initial part of the curve shown on Fig. 5 presents an averaged pattern that is in good agreement with that observed on the curves of stress versus deformation obtained through tensile tests performed on rabbit ACL (Panjabi *et al.*, 2001). The accentuated drop of both load and stress curves observed on Figs. 4 and 5 are very probably due to the sliding between the specimen and the metallic surface. In this part of the experimental tests, the loading level has been low enough to keep the behavior of the specimen within the elastic regime.

Secondly, the four specimens prepared with the femoral and tibial bone ends are mounted on the testing machine to obtain the curves of excitation versus response for the anterior cruciate ligament. Four elongation time rates are used in the tensile tests; however, low adherence on the contact surface between the screw and the bone impedes to perform entirely the tensile tests at speeds of 0.3 mm/s and 0.83 mm/s. As the axial load increases during the test at these two speeds, the sliding between screw and bone begin. The gripping technique using the end bones with screws demonstrate to be efficient only at low testing elongation time rates. Figures 6 and 7 depict the axial load versus displacement curve and the stress versus strain time rate curve, respectively, for the ACL ligaments tested at elongation time rates of 0.017 mm/s and 0.17 mm/s. The stress-deformation gradient curve pattern shown in Fig. 7 agrees well with that presented by Pioletti (1997) for the curves obtained for soft tissues comprised of collagen fibers.

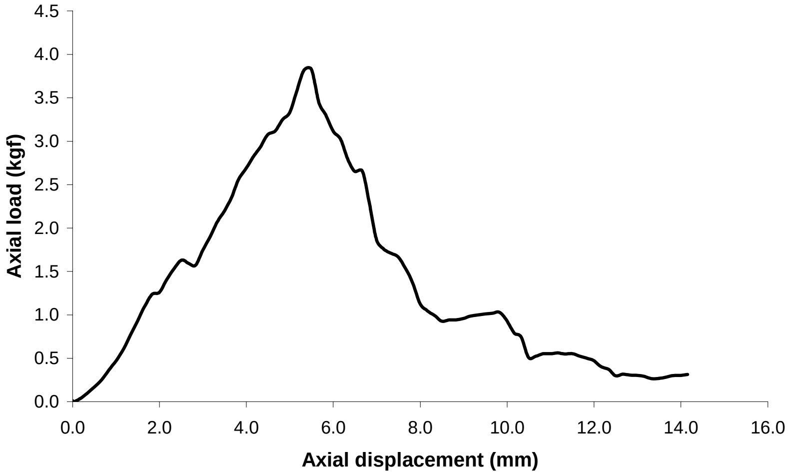

Figure 4. Curve of axial load versus displacement for canine ACL specimens without the bone ends at elongation time rate of 0.17 mm/s.

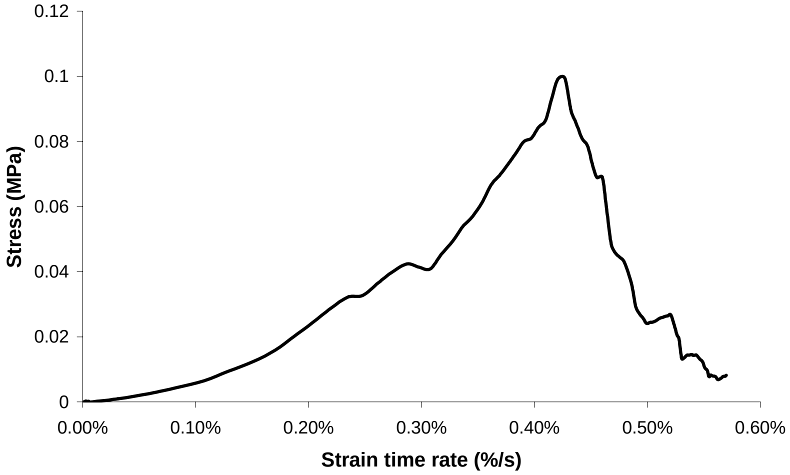

Figure 5. Stress-strain time rate curve of the ACL specimens without the bone ends elongation time rate of 0.17 mm/s.

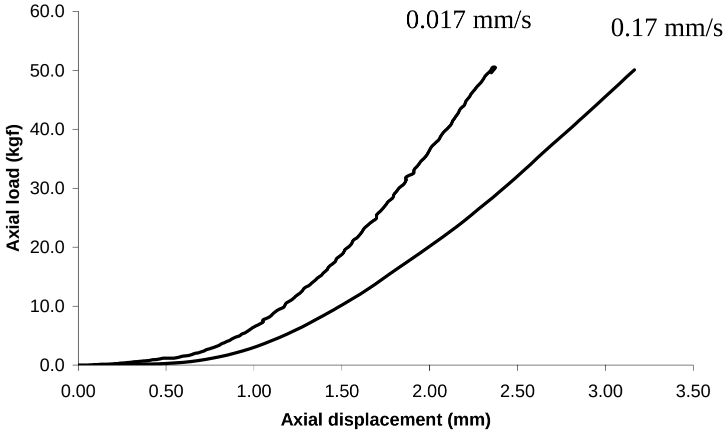

Figure 6. Curves of axial load versus axial displacement for canine ACL specimens with bone ends at two values of elongation time rate.

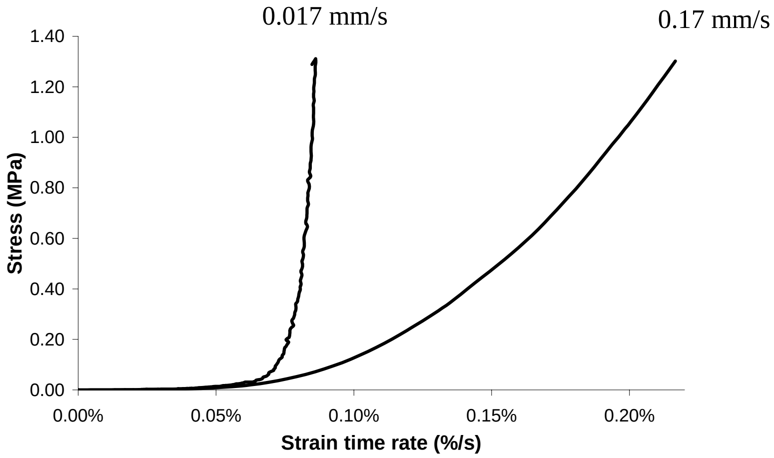

Figure 7. Curves of stress versus strain time rate for canine ACL specimens with bone ends at two values of elongation time rate.

4. Discussion about Methodologies and Analysis

Three experimental aspects of the tensile tests with soft tissues comprised of collagen fibers can be highlighted. Firstly, the gripping technique used to fix the soft tissues onto the testing machine plays a fundamental role on the experimental studies developed about the mechanical properties of this kind of material. No paper in the technical literature has dealt with this matter properly. Methodological basis for choosing the gripping technique more appropriate to describe the tissue behavior deserves a more detailed comparative analysis of all techniques available. There is no well established norm or standard for the tensile tests of soft tissues. The results presented by many authors, who have been working on the biomechanical characterization of structures constituted of collagen fibers, present an expressive discrepancy and the issues related to the specimen preparation and conservation are up to a judicious scrutiny from researchers in this field.

Secondly, measurements of dimensional characteristics of soft tissues do not follow standardization. In the vast majority of the published material on the biomechanical literature, dimensions for the ACL specimens, from different animals, have not been provided. Naturally, the ACL specimens can vary substantially for a same species. Furthermore, measurements of lengths and cross-section dimensions for soft tissue specimens are performed based on different methodologies and instruments. The third experimental aspect is associated with the elongation time rate for testing collagen structures. The curves of stress are presented against deformation as well as against deformation gradient at a unique stretching speed or at various loading speeds. That is, there is not a clear elongation time rate to test ligament tissues.

The technical difficulties associated with the gripping technique for collagen structures, which consist of very soft material with complex geometry, are superficially demonstrated in this paper. Figures 1 and 2 show pictures of the two different grippers used in this work. The first gripper, which employs two metallic plates pressing the ligament ends, has several limitations, mainly due to the expressive sliding observed during the tensile tests even at low loads (about 5 kgf) and to the difficulties of aligning the collagen fibers along the axial direction. The second gripper, which uses the bone ends with screws, is more efficient than the former one. The sticking property of the adhesive employed to attach the screw on the end bone is an important issue when this gripper is used. The alignment of the ACL fibers with the axial load is achieved when the angle between the femoral and tibial end bones is approximately 120^{o}. To guarantee this alignment, the screws must be carefully attached to the end bones, as shown in Fig. 3. Even though all precautions are taken during the tensile tests with the second gripping technique, some sliding takes place for high loads (above 50 kgf).

The curves of axial load and normal stress shown in this work provide some insights into the mechanical behavior of canine ACL during tensile tests. The loading speed dependence of the stress curve is clearly shown in Fig. 7. The tangent modulus for the ligaments, which can be estimated by the slope of the stress-strain curves, varies strongly as the elongation time rate changes. At low loading speeds, the tangent modulus is almost constant, as it can be seen from the stress-strain time rate curve at 0.017 mm/s. On the other hand, this modulus varies significantly when the testing speed increases, as it is shown on the stress-strain time rate curve at 0.17 mm/s.

In order to eliminate most of the technical difficulties presented by the two grippers used in this work, a third gripping technique can be proposed. Figure 8 shows a schematic drawing of a new gripper, which is under development to test the ACL specimens with end bones. This design allows testing specimens of different sizes at several inclination angles between bone end centerlines. The upper part of this gripper has a special screw to allow some adjustments on the alignment. The end bones are placed inside the two metallic hollow cylinders, which have two screws to attach them.

5. Final remarks

The present study provided information on a protocol to test ligaments. Based on the described experiments, some conclusive remarks can be drawn: (a) Testing without separating the ligament from the bone were is is attached provides a simpler and more effective setup; (b) Strain rate values should be established a priori, according to the problem under study, due to the sensitivity of the results to this parameter; (c) Adequate positioning of the specimen during the test can be obtained with a variation of the grip used in this study, as proposed in item 3; (d) For low stress levels, the tests provided information on the behavior of the ligament which s consistent with the available data from the literature.

Further study is currently being performed by the authors for a complete characterization of the viscoelastic behavior of the canine ligament for certain strain rates.

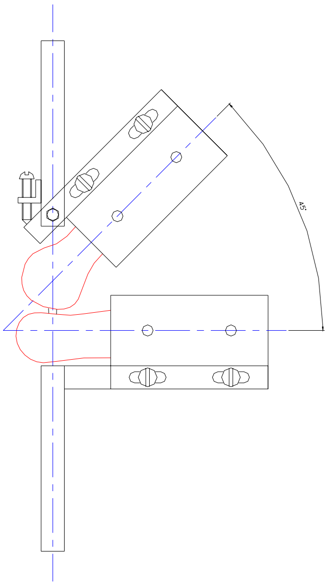

Figure 8. Schematic drawing of a new gripper for testing ACL specimens attached to the end bones.

6. References

Bertolan, A., P., 2000, “Biomechanical Properties of fascia lata and cranial cruciate ligament of dogs”, M.Sc. Thesis, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil, 38p.

Kapanji, A., I., 2000, “Physiology Articulate”, Ed. Paramericana, São Paulo, Brazil, 280 p.

Miyasaka, K.C., Daniel, D. M., Stone, M.L. and Hirshman, P., 1991. “The Incidence of Knee Ligament Injuries in the General Population”, American Journal of Knee Surgery, Vol. 4, pp. 3-8.

Nakano, H., Yasuda, K., Tohyama, H., Yamanaka, M., Wada, T. and Kaneda, K., 2000, “Interference Screw Fixation of Doubled Flexor Tendon Graft in Anterior Cruciate Ligament Reconstruction - Biomechanical Evaluation with Cyclic Elongation”, Clinical Biomechanics, Vol. 15, pp. 188-195.

Panjabi, M. M. and Courtney W.T., 2001, “High-Speed Subfailure of Rabbit Anterior Cruciate Ligament: Changes in Elastic, Failure and Viscoelastic Characteristics”, Clinical Biomechanics, Vol. 16, pp. 334-340.

Pioletti, D.P., Rakotomanana, L. R., Benventurti, J. F. and Leyvraz, P. F., 1998, “Viscoelastic Constitutive law in large deformations: application to human knee ligaments and tendons”, J. of Biomechanics, Vol. 31, pp. 753 – 757.

Pioletti, D.P., 1997, “Viscoelastic Properties of soft tissues: application of knee ligaments and tendons”, Ph.D. Dissertation, Federal Polytechnic School of Lausanne, Switzerland, 106 p.

Yamamoto, E., Tokura, S., Yamamoto, N., Hayashi, K., 2000, “Mechanical Properties of Collagen Fascicles From in Situ Frozen and Stress-Shielded Rabbit Patellar Tendons”, Clinical Biomechanics, Vol. 15, pp. 284 – 291.

7. Responsibility notice

The authors are the only responsible for the printed material included in this paper.

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