Article published online: 2022-06-06

Monoaxial Mechanical Tests on Porcino Knee Ligaments*

Ensaios mecânicos monoaxiais nos ligamentos do joelho porcino

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Rev Bras Ortop 2023;58(1):173–178.

Abstract

The failure of ligament reconstruction has different risk factors, among which we can highlight the period before its incorporation, which is a mechanically vulnerable period. Loss of resistance over time is a characteristic of living tissues. Dissection with bone insertions of the cruciate ligaments of animal models is not described; however, it is essential for monoaxial assays to extract information from tests such as relaxation. The present work describes the dissection used for the generation of a test body for the performance of nondestructive tests to evaluate the mechanical behavior. We performed dissection of four porcino knee ligaments, proposing a dissection technique for the cruciate ligaments with bone inserts for comparison with collateral ligaments. The ligaments were submitted to relaxation tests and had strain gauges placed during the tests. The results showed viscoelastic behavior, validated by strain gauges and with a loss over time; with some ligaments presenting with losses of up to 20%, a factor to be considered in future studies. The present work dissected the four main ligaments of the knee demonstrating the posterior approach that allows maintaining their bone insertions and described the fixation for the monotonic uniaxial trials, besides being able to extract the viscoelastic behavior of the four ligaments of the knee, within the physiological limits of the knee.

Keywords

- dissection
- biomechanical phenomena
- knee joint
- tensile strength
- models, animal

* *Work developed in the Centro Federal de Educação Tecnológica Celso Suckow da Fonseca (CEFET/RJ), Rio de Janeiro e Instituto Nacional de Traumatologia e Ortopedia (INTO), Rio de Janeiro, RJ, Brazil.*

received
November 20, 2021
accepted
March 4, 2022
article published online
June 6, 2022

DOI https://doi.org/10.1055/s-0042-1748964.
ISSN 0102-3616.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Resumo

A falha da reconstrução ligamentar tem diferentes fatores de risco, dentre os quais podemos destacar o período antes da sua incorporação, o qual configura um período mecânico vulnerável. A perda de resistência ao longo do tempo é uma característica dos tecidos vivos. A dissecção com as inserções ósseas dos ligamentos cruzados de modelos animais não é descrita; todavia, para os ensaios monoaxiais, é fundamental extrair as informações de ensaios como os de relaxação. O presente trabalho realiza a descrição da dissecção utilizada para a geração de corpo de prova para a realização de ensaios não destrutivos para avaliar o comportamento mecânico. Realizamos dissecção de quatro ligamentos de joelho porcino, propondo uma técnica de dissecção para os ligamentos cruzados com as inserções ósseas para comparação com os colaterais. Os ligamentos foram submetidos a testes de relaxação e foram colocadas *strain gauges* durante os testes. Os resultados mostraram comportamento viscoelástico, validado pelas *strain gauges* e com uma perda ao longo do tempo, sendo que, em alguns ligamentos, as perdas chegaram a até 20%, fator este a ser considerado em trabalhos futuros. O presente trabalho dissecou os quatro principais ligamentos do joelho, demonstrando a abordagem posterior que permite manter as suas inserções ósseas e descrevendo a fixação para os ensaios uniaxiais monotônicos, além de ter conseguido extrair o comportamento viscoelástico dos quatro ligamentos do joelho dentro dos limites fisiológicos do joelho.

Palavras-chave
- dissecção
- fenômenos biomecânicos
- articulação do joelho
- resistência a tração
- modelo animal

Introduction

The failure of ligament reconstruction has different risk factors, among which we can highlight the period before its incorporation, which is a mechanically vulnerable period.[1]

Knee ligament injuries are high-frequency lesion due to sports practice, among which we highlight the injuries of the four main ligaments: the medial collateral ligament, (MCL), the lateral collateral ligament (LCL), the anterior cruciate ligament (ACL), and the posterior cruciate ligament (PCL).

Loss of resistance over time is a characteristic of living tissues; for example, what happens with the viscoelastic behavior of each structure. If there is no ligament reconstruction in this behavior, this may be the cause of failure, even with normal resonances, as described by Ekdahl et al.,[2] resulting in an inadequate redistribution of efforts in knee structures.

Several studies performed limit tests of ligament resistance,[3–5] mimicking ligament or graft ruptures. However, there is no work in the literature that studied ligaments in isolation within the physiological range of deformation and extracted the information of this behavior specially in anterior and posterior cruciate ligaments.

Studies can be destructive or nondestructive, although limit tests of the destructive type are the most found in the literature. However, for the extraction of ligament behaviors and to simulate behavioral situations that occur within the normal physiology, some authors suggest performing the tests within the physiological limits of deformations (from 3 to 6%).

Dissection with the bone insertions of the cruciate ligaments of animal models is not described; however, it is essential for monoaxial essays to extract information from tests such as relaxation, as proposed by Duenwald et al.[6] The present work describes the dissection of the four ligaments used for the generation of a test body and the performance of nondestructive tests (relaxation) in monoaxial traction tests, with emphasis on the ACL of the porcine knee.

Material and Method

The study protocol was approved by the Ethics Committee on the Use of Animals as prescribed by the Regulation of Sanitary and Industrial Inspection of Animal Products (MAPA, in the Portuguese acronym).

The four pig knee ligaments (MCL, LCL, ACL, and PCL) were dissected with their bone insertions as described below.

Dissection was performed starting with the collateral ligaments, the first being the medial collateral due to its ease of approach, because it is very superficial. The bone inserts were maintained with a 30-mm length at each end to serve as fixation points. In each bone fragment (proximal and distal), a 6-mm hole was made to install the connecting pins with the INSTRON material testing machine (Instron 5966, Norwood, Massachusetts, USA). After removal of the collaterals, the cruciate ligaments are removed in sequence. Dissection should be performed through the posterior view (►**Fig. 1**).

The cut is performed between the posterior and anterior cruciates at the femoral level, through a posterior approach (►**Fig. 2**).

After separation of the proximal extremity, the PCL is removed with the bone fragment in the proximal tibia. This removal allows the visualization of the tibial insertion of the ACL, allowing the removal of the tibia with the bone fragment (►**Fig. 3**).

We made a modification in the technique of removal of the cruciate ligaments proposed in the article by Skelley

Fig. 1 Posterior vision of the knee with osteotomy of the tibial insertion.

et al.,[7] who removed the ligaments without the bone fragments.

To date, there is no description of removals of the cruciate ligaments with their bone insertions. The removal with the bone fragments of the ACL inserts prevents the slipping of the ligament specimens in the claws, providing an adequate reading of the viscoelastic behavior of the ligament within physiological limits.

Fig. 3 Osteotomy of the femur between the cruciate ligaments.

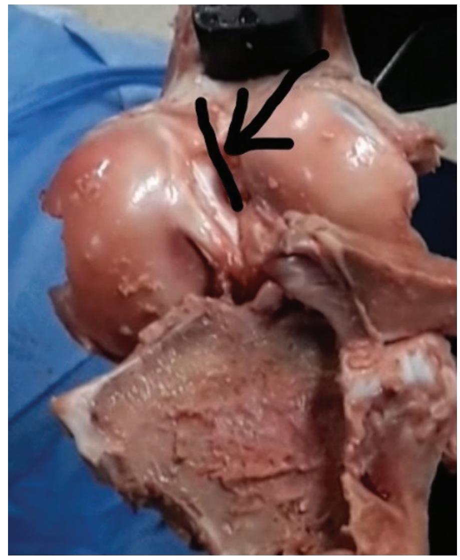

Fig. 2 Visualization of the osteotomy site after medialization of the bone plug of the posterior cruciate ligament.

On the day of dissection, a model was thawed at air-conditioned room temperature (22°C) for 5 hours. Then, it was dissected isolating the four main ligaments of the knee (ACL, PCL, MCL, and LCL), maintaining their bone insertions with 30 mm of bone fragment. A 6-mm hole was made, cross-sectional to each bone insert, for pin coupling for correct positioning in the material testing machine. The pig ligament specimens were then placed in plastics and soaked in gauze with 0.9% saline and kept until their testing, which was carried out on the same day.

The models were then placed in the INSTRON material testing machine of the LADES laboratory of CEFET/RJ with a load cell of 1 kN. At an initial moment, the ligament is subjected to an axial load of 10 N during a period of 5 minutes to reach its original length (►**Fig. 4**), as described by Troyer et al.[8]

Regarding the relaxation tests, the three-step protocol proposed by Duenwald et al. was used[6] with maximum physiological deformations of 6% and with small 3% deformations being usually found, as proposed by the work of Gardiner et al.[9]

The proposed protocol, known as Hill-Valley-Hill, served to simulate behavior in an in vivo situation. It is characterized by performing deformations within the expected physiological limit for adequate joint mobility, ranging between 3% (lower limit) and 6% (upper limit) over a period of 100 seconds and done in three stages: one performed as a relaxation test (6%), followed by recovery (3%) and by another relaxation test (6%).[6] Then, data from the behavioral results over time were extracted, with the objective of characterizing the viscoelastic behavior of the ligaments.

Fig. 4 Ligament coupled with strain gauge on the Instron.

Results

The results of the tests performed on the four ligaments are shown in the force versus time graphs, in which force signals were obtained from the INSTRON load cell (►**Fig. 5**).

When analyzing the behavior of the MCL, there was a loss of < 10% of its load in the relaxation test in the 1st cycle, attenuating in the 2nd cycle. The LCL behavior also decreased < 10%; however, the 2nd cycle still showed a loss of load, but of lower decrease, stabilizing with 100 seconds.

The loss by the viscous component of the PCL was evident and the peak in deformation at 6% was of 160 N and showed a fall in the 2nd cycle and it was of a lower value than that of the 1st cycle, demonstrating its viscoelastic behavior.

The loss by the viscous component of the anterior cruciate ligament was evident and the 120 N peak in deformation at 6% showed a drop in the 2nd cycle, being less distinct than that of the 1st cycle, which reached values < 100 N, with an absolute difference of 20 N (which was greater than that of the 2nd cycle).

The results showed viscoelastic behavior with a loss over time, which is a factor to be considered in future studies.

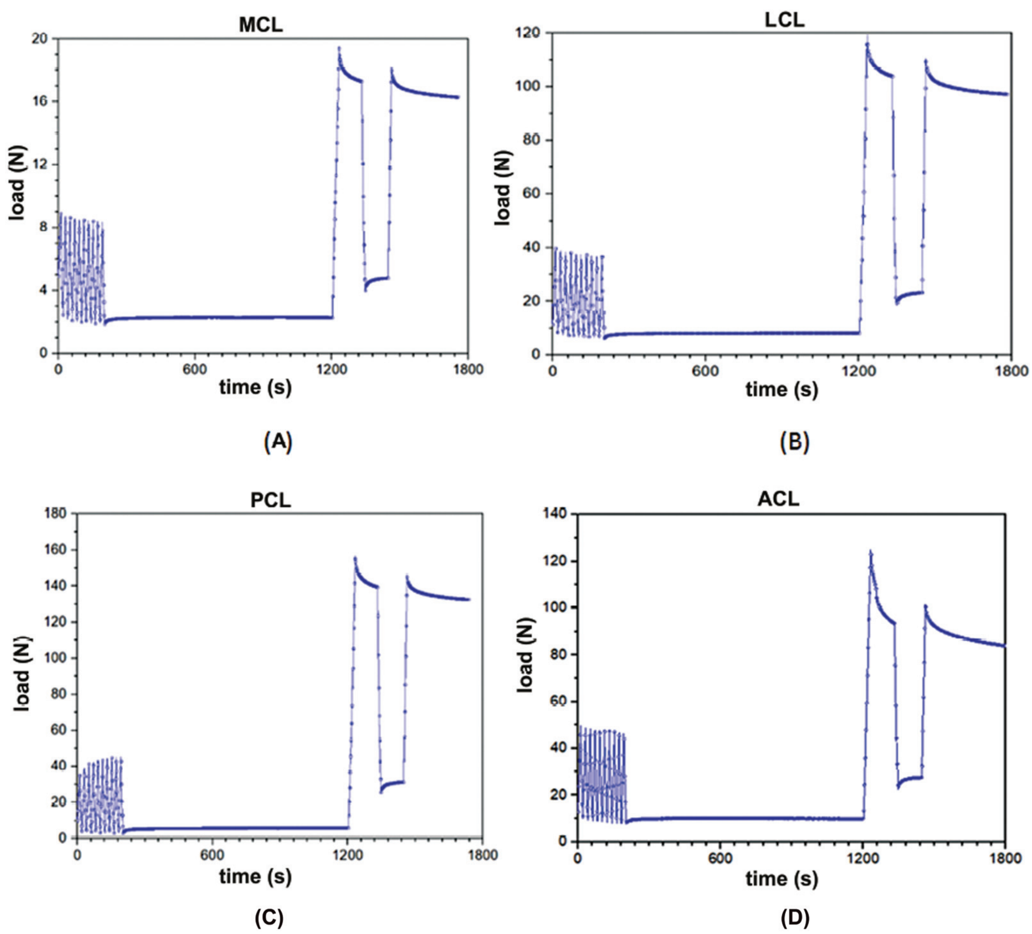

Fig. 5 (**A**) Graph of the force reading x medial collateral ligament time; (**B**) Graph of the strength reading x time of the lateral collateral ligament; (**C**) Graph of the force reading x time of the posterior cruciate ligament; (**D**) Force reading graph x anterior cruciate ligament time.

Final Comments

The present technical note dissected the four main ligaments of the knee demonstrating the step by posterior vision, extracting with the ligaments and described the fixation for the monotonic uniaxial essays and was able to extract the behavior of the viscoelastic pattern of the four knee ligaments, within the physiological limits of the knee, through adequate specimens obtained with their bone insertions. All ligaments tested showed loss of load over time. These findings point to the importance of new experimental and ligament trials and tendon options for reconstruction, including serving as a reference for graft choices with ligament-like behaviors and simulation models.[10]

Financial Support
The present study did not receive any financial support from public, commercial, or not-for-profit sources.

Conflict of Interests
The authors have no conflict of interests to declare.

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