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Repeatability of experimental procedures to determine mechanical behaviour of ligaments

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The aim of this study was to investigate intra- and interspecimen repeatability of an experimental procedure, which determines elastic and viscoelastic properties of knee ligaments. The collateral ligaments from sheep were used and the repeatability was evaluated in terms of the coefficient of variation.

The results indicated a good intraspecimen repeatability (the coefficient of variation generally less than 5%), whereas the interspecimen repeatability was lower (coefficient of variation of about 50%). In conclusion, since the intraspecimen coefficient of variation was low the test procedure was assumed to be repeatable.

Key words: *soft tissue, ligaments, mechanical properties, experimental procedure*

1. Introduction

The development of functional musculoskeletal computer models requires accurate characterizations of the tissues involved. One of those tissues are the ligaments. A great number of studies have been done to characterise different aspects of ligaments, e.g. elastic behaviour, stress and strain relaxation properties [1]–[7]. Furthermore, several studies have been aimed to determine the accuracy of models to predict experimental tests [8]–[10]. Surprisingly, to the authors' knowledge, no study has been carried out explicitly to assess intra- and interspecimen test repeatability. Good test repeatability is essential since it is indicative of the procedure quality. This is a prerequisite if the experimental data have to be used to, in the end, develop a well functioning musculoskeletal model.

The aim of this study was to investigate, in terms of coefficient of variation, the intra- and interspecimen repeatability of two types of test: 1) elastic test; 2) stress relaxation test on knee ligaments.

2. Materials and methods

2.1. Animals

Ovine collateral ligaments were selected as the in vitro model for this study. Sheep is commonly used as an animal model to develop new surgical techniques [11]–[13]. Whole sheep knees with 5 cm of tibia and femur were obtained from a local butcher. Since the animals were killed for alimentary purpose, it was not necessary to obtain an ethical authorisation. Care was taken to ensure that the collagen fibres of the soft tissue, both of the free length and at the insertion points, were not damaged during retrieval. The knees were wrapped in saline-solution (0.9% NaCl) soaked gauze and frozen at –20°C directly after harvesting. The effect of freezing

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ligaments in this manner prior to biomechanical testing has been established in a previous study [14].

2.2. Specimen preparation

Knee samples were thawed in saline solution at room temperature before specimen preparation and testing. Each knee was cleaned of all unwanted soft tissue except for the desired ligaments, i.e. the collateral ligaments were left attached to the bone. To isolate the ligaments, small pieces of bone at the insertion point of the ligaments were cut of. All excessive soft tissue attached to the ligament was removed. The bone piece at one end of the ligament was lowered into a parallelepipedal mould with the ligament aligned vertically. The mould was filled with acrylic resin. Care was taken to make sure that the cement did not cover the insertion point. The acrylic resin block was then removed from the mould and this procedure was repeated for the other end of the ligament. Through the whole specimen preparation procedure the ligament was wrapped in gauze soaked with saline solution to make sure that the soft tissue did not dry.

2.3. Mechanical testing

Two different experimental tests were implemented, an elastic test and a stress relaxation test. The former was designed to determine the toe elastic modulus, i.e. the elastic response of the tissue at slight deformation, and the linear elastic modulus, i.e. the elastic response of the tissue at severe deformation. The latter was designed to investigate the stress relaxation, i.e. the changes in stress with time holding the specimen at a certain strain. Furthermore, since soft tissue behaviour may depend on the speed of deformation, two different strain rates were selected for the tests: 1) low (0.1 s^{-1}) and high (1.0 s^{-1}). Both strain rates are within the physiological range, the slow one representing a daily activity such as walking [15] and the high one – a sporty activity such as running [16]. The experimental procedures included testing only in the elastic range of the soft tissue. Test repetitions were done both on the same specimen and on groups of specimens to assess both intra- and interspecimen repeatability. In the case of intraspecimen tests, the ligament was left at least 30 min unloaded to recover between repetitions.

The specimen was fixed with two custom-made clamps (clamping area of ~ 10×30 mm^2) onto the material-testing machine (858 Mini Bionix MTS systems corp., Minneapolis, MN, USA): one clamp was attached to the load cell and the other one to the actuator. The specimen was fixed at the levels of the acrylic resin. Care was taken to ensure the alignment of the specimen with the machine axis. Tests were performed at room temperature (about 20 °C) since soft tissues maintained at 37 °C dry faster. All specimens were slowly preloaded to 5 N (0.1 mm/s displacement rate) to remove ligament slack [5]. Width and thickness were measured in five points along the specimen. The free length between the points of ligament insertion into the bone was also measured. The length measurement was used to calculate the crosshead displacement rate, which assured the desired tissue strain rate. The soft tissue was wrapped in saline-soaked (0.9% NaCl) gauze. An extensometer (Mod. 634.31F-24, MTS systems corp., Minneapolis, MN, USA, gauge length: 19 mm) was attached to the central part of the ligament to measure its elongation. Both arms of the extensometer were equipped with a thin needle, which were pinned into the tendon (see figure 1). The specimen was preconditioned by displacement-controlled cycling between 0 and 2% strain for twenty cycles at 0.5 Hz to remove any crimping in the tendon fibrils due to prolonged storage in a fixed position [3].

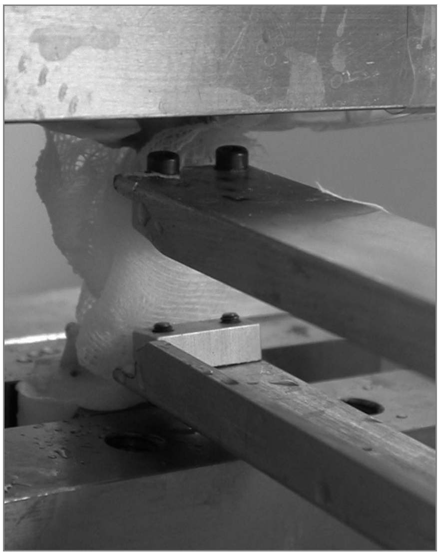

Fig. 1. Set-up of mechanical test

2.3.1. Elastic test

All specimens were loaded in displacement control to 5% strain [17] while force and extensometer displacement were registered. Stress was determined as force divided by average cross section, while strain as extensometer displacement divided by gauge length. Toe modulus was defined as the initial linear part of the stress–strain curve, while linear elastic modulus was defined as the linear part at high deformation. Ten specimens were tested for each strain rate. In four cases, for both strain rates, five test repetitions were done on the same specimen.

2.3.2. Stress relaxation test

All specimens were loaded in displacement control to 5% strain [17] and then held at that strain for 15 min while force and time were registered. Stress was calculated and normalised and thereafter a plot of normalised stress versus time was done. Ten specimens were tested for each strain rate. In four cases, for both strain rates, five test repetitions were done on the same specimen.

2.4. Statistical analysis

The coefficients of variation, calculated for the data collected from one specimen and from a group of specimens, were used to quantify the intraspecimen and interspecimen repeatability, respectively. In the case of toe or linear elastic modulus, the values were compared directly, since this is the value, which represents the physical characteristics of the tissue. Conversely, in the case of the stress relaxation tests, the distance between the curves at each registered time point was used. In the latter case, the experimental curves were compared to delete any possible effect due to the equation selected to fit the data.

3. Results

3.1. Intraspecimen repeatability

The results from the elastic tests are summarised in table 1. All toe and linear elastic moduli had the coefficients of variation of less than 5%, except for those where some noise was observed in the strain data. This caused in the worst case an increase in the coefficient of variation up to 18%.

Table 1. Intraspecimen repeatability of elastic tests

Specimen	Strain rate	Coefficient of variation	
		Toe modulus	Linear elastic modulus
Lig 1	$0.1\ s^{-1}$	4.6%	1.4%
Lig 2		18.1% *	6.5% *
Lig 3		1.1%	4.2%
Lig 4		2.5% *	7.0% *
Lig 11	$1\ s^{-1}$	0.6%	1.9%
Lig 12		4.3%	2.4%
Lig 13		2.0%	1.4%
Lig 14		3.7% *	8.8% *

* In at least one test repetitions, some noise in strain measurements did not permit the accurate determination of toe and linear elastic modulus.

Table 2. Intraspecimen repeatability of stress relaxation tests

Specimen	Strain rate	Coefficient of variation
Lig 21	$0.1\ s^{-1}$	7.5%
Lig 22		4.3%
Lig 23		6.1%
Lig 24		8.7%
Lig 31	$1\ s^{-1}$	1.4%
Lig 26		1.3%
Lig 27		1.2%
Lig 28		3.5%

An example of the stress relaxation curves from one specimen can be seen in figure 2. The maximum coefficient of variation for the distances between the curves of relaxation was always less than 10% for the slow strain rate and less than 5% for the fast one (see table 2).

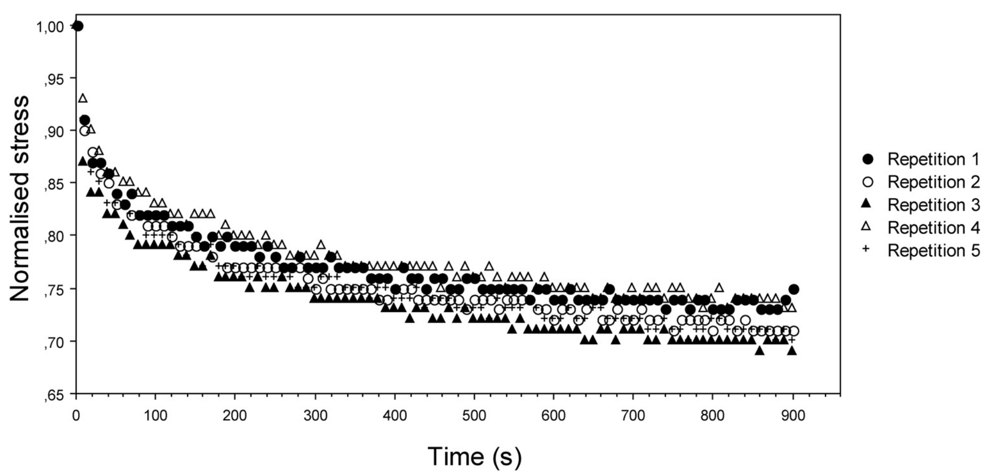

Fig. 2. Normalised stress versus time curves of five intraspecimen repetitions

3.2. Interspecimen repeatability

Six specimens (three for each strain rate) were damaged during specimen preparation or testing and were hence excluded from the analysis. The coefficients of variation for toe modulus were 51% and 59%, for slow and fast strain rates, respectively, while the coefficients of variation for linear elastic modulus were 47% for the slow strain rate and 52% for the high one. Furthermore, the maximum coefficients of variation for the distances between the ligament curves of relaxation were 25% and 21% for the slow and fast strain rates, respectively.

4. Discussion

The aim of this study was to determine the intra- and interspecimen repeatability of an elastic test and a stress relaxation test. Sheep ligaments were used since the aim was not to determine the mechanical characteristics but to investigate the procedure quality. Collateral ligaments were harvested from sheep knees. An elastic test was done to assess the toe and linear elastic moduli of the soft tissue. Furthermore, a stress relaxation test was carried out to obtain the viscoelastic properties of the tissue. Test repetitions were done both on single specimens and on groups of specimens.

The intraspecimen tests had an overall coefficient of variation of less than 10%. The interspecimen tests had a higher coefficient of variation, ranging from 21% (the fast strain rate, stress relaxation test) to 59% (the fast strain rate, toe modulus test).

To the authors' knowledge, no study has been conducted to assess the intraspecimen repeatability of elastic and stress relaxation tests on ligaments. However, the coefficient of variation of less than 10% seems to be a good result. From a living material, such as ligaments, a higher repeatability should not be expected.

Interspecimen repeatability can in many cases be calculated from the results in the literature [3], [4], [6], [17], [18]. A coefficient of variation ranging from 11% [6] to 50% [3] can be found. The results of the present study are close to this range. The high value might be explained by the fact that the sheep were obtained from a local butcher, hence nothing was known about age, sex, breed, possibilities to move, etc. All of those seem to be important factors for soft tissue properties [3], [19]–[21]. Referring to the experimental procedure, the measurements of the cross-section area are not optimal. The use of a non-contact measuring technique, similar to those which have been used to define one cross-section [22], can define more accurately the 3D geometry of the ligament and should reduce the interspecimen coefficient of variation.

5. Conclusion

In conclusion, since the intraspecimen repeatability was satisfactory, the measurements of force and displacement can be assumed to be accurate. Conversely, to improve interspecimen repeatability further studies should be done, considering a non-contact measuring device to define the 3D geometry of the specimens.

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References

[1] AMIS A.A., BULL A.M., GUPTE C.M., HIJAZI I., RACE A., ROBINSON J.R., *Biomechanics of the PCL and related structures: posterolateral, posteromedial and meniscofemoral ligaments*, Knee Surg. Sports Traumatol. Arthrosc., 2003, 11(5), 271–281.

[2] BONIFASI-LISTA C., LAKE S.P., SMALL M.S., WEISS J.A., *Viscoelastic properties of the human medial collateral ligament under longitudinal, transverse and shear loading*, J. Orthop Res., 2005, 23(1), 67–76.

[3] CHANDRASHEKAR N., MANSOURI H., SLAUTERBECK J., HASHEMI J., *Sex-based differences in the tensile properties of the human anterior cruciate ligament,* J. Biomech., 2006, 39(16), 2943–2950.

[4] ROBINSON J.R., BULL A.M., AMIS A.A., *Structural properties of the medial collateral ligament complex of the human knee,* J. Biomech., 2005, 38(5), 1067–1074.

[5] KOH S.W., CAVANAUGH J.M., LEACH J.P., ROUHANA S.W., *Mechanical properties of the shoulder ligaments under dynamic loading*, Stapp Car Crash J., 2004, 48, 125–153.

[6] MOON D.K., WOO S.L., TAKAKURA Y., GABRIEL M.T., ABRAMOWITCH S.D., *The effects of refreezing on the viscoelastic and tensile properties of ligaments*, J. Biomech., 2006, 39(6), 1153–1157.

[7] STABILE K.J., PFAEFFLE J., WEISS J.A., FISCHER K., TOMAINO M.M., *Bi-directional mechanical properties of the human forearm interosseous ligament*, J. Orthop. Res., 2004, 22(3), 607–612.

[8] ABRAMOWITCH S.D., WOO S.L., *An improved method to analyze the stress relaxation of ligaments following a finite ramp time based on the quasi-linear viscoelastic theory*, J. Biomech. Eng., 2004, 126(1), 92–97.

[9] DEFRATE L.E., LI G., *The prediction of stress relaxation of ligaments and tendons using the quasi-linear viscoelastic model*, Biomech. Model Mechanobiol., 2007, 6(4), 245–251.

[10] MOMMERSTEEG T.J., HUISKES R., BLANKEVOORT L., KOOLOOS J.G., KAUER J.M., *An inverse dynamics modeling approach to determine the restraining function of human knee ligament bundles*, J. Biomech., 1997, 30(2), 139–146.

[11] FUNAKOSHI Y., HARIU M., TAPPER J.E., MARCHUK L.L., SHRIVE N.G., KANAYA F., RATTNER J.B., HART D.A., FRANK C.B., *Periarticular ligament changes following ACL/MCL transection in an ovine stifle joint model of osteoarthritis*, J. Orthop. Res., 2007, 25(8), 997–1006.

[12] WALSH W.R., STEPHENS P., VIZESI F., BRUCE W., HUCKLE J., YU Y., *Effects of low-intensity pulsed ultrasound on tendon-bone healing in an intra-articular sheep knee model*, Arthroscopy, 2007, 23(2), 197–204.

[13] GERBER C., SCHNEEBERGER A.G., BECK M., SCHLEGEL U., *Mechanical strength of repairs of the rotator cuff*, J. Bone Joint Surg. Br., 1994, 76(3), 371–380.

[14] WOO S.L., ORLANDO C.A., CAMP J.F., AKESON W.H., *Effects of postmortem storage by freezing on ligament tensile behavior*, J. Biomech., 1986, 19(5), 399–404.

[15] WREN T.A., YERBY S.A., BEAUPRE G.S., CARTER D.R., *Mechanical properties of the human Achilles tendon*, Clin. Biomech. (Bristol, Avon), 2001, 16(3), 245–251.

[16] SCHENCK R.C. Jr., KOVACH I.S., AGARWAL A., BRUMMETT R., WARD R.A., LANCTOT D., ATHANASIOU K.A. *Cruciate injury patterns in knee hyperextension: a cadaveric model*, Arthroscopy, 1999, 15(5), 489–495.

[17] CLAVERT P., KEMPF J.F., BONNOMET F., BOUTEMY P., MARCELIN L., KAHN J.L., *Effects of freezing/thawing on the biomechanical properties of human tendons*, Surg. Radiol. Anat., 2001, 23(4), 259–262.

[18] SUGITA T., AMIS A.A., *Anatomic and biomechanical study of the lateral collateral and popliteofibular ligaments*, Am. J. Sports Med., 2001, 29(4), 466–472.

[19] MATSUMOTO F., TRUDEL G., UHTHOFF H.K., BACKMAN D.S., *Mechanical effects of immobilization on the Achilles' tendon*, Arch. Phys. Med. Rehabil., 2003. 84(5), 662–667.

[20] WOO S.L., OHLAND K.J., WEISS J.A., *Aging and sex-related changes in the biomechanical properties of the rabbit medial collateral ligament*, Mech. Ageing Dev., 1990, 56(2), 129–142.

[21] KOMATSU K., YAMAZAKI Y., YAMAGUCHI S., CHIBA M., *Comparison of biomechanical properties of the incisor periodontal ligament among different species*, Anat. Rec., 1998, 250(4), 408–417.

[22] MOON D.K., ABRAMOWITCH S.D., WOO S.L., *The development and validation of a charge-coupled device laser reflectance system to measure the complex cross-sectional shape and area of soft tissues*, J. Biomech., 2006, 39(16), 3071–3075.