Chapter 5

In-situ mechanical behavior and the slack length of the anterior cruciate ligament at multiple knee flexion angles

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Abstract

This study evaluated the in-situ tensile behavior and slack length of the anterior cruciate ligament (ACL) at various knee flexion angles. In four cadaver knees the ACL was released at the tibial insertion, after which it was re-connected to a tensiometer. After pre-tensioning (10N) the ACL in full extension, the knee was flexed (from 0° to 150°) with 15° increments, at which flexion angles the ACL tension was measured. At each angle the ACL was subsequently elongated and shortened under displacement control, while measuring the ACL tension. In this manner, the pretension or the slack length, and the tensile behavior of the ACL were measured.

All ACL’s displayed a higher tension at low (0°-45°) and high (120°-150°) flexion angles. The ACL slack length depended on flexion angle, with the highest slack length found at 75° and 90° of flexion. Additionally, the ACL stiffness also varied with flexion angle. The ACL was stiffer at low and high flexion angels than at intermediate flexion angles. In general, the ACL was stiffest at 150°, and most compliant at 90° flexion. The results of this study contribute to understanding the mechanical behavior of the ACL in-situ, and may help tuning and validating computational knee models studying ACL function.

Keywords:
ACL, displacement, in-situ, knee flexion, slack length, tension.

1. Introduction

The anterior cruciate ligament (ACL) fulfills an important role in maintaining knee stability, especially for restraining anterior tibial translation (Sanchez et al., 2006; Woo et al., 2006; Dargel et al., 2007). It is also the most frequently ruptured ligament in the knee (Song et al., 2004; Daniel et al., 1994; Griffin et al., 2000). Surgical reconstruction of the ACL is a common intervention to treat disability or chronic instability of the knee (Beynnon et al., 2002). Unfortunately, in some cases the results are still unsatisfactory, resulting in the development of secondary injury, or progressive degeneration of the injured knee joint (Dargel et al., 2007), and failed restoration of the physiological characteristics of a native ACL (Beasley et al., 2005).

To analyze the function of the ACL, and to optimize the results of surgery, three-dimensional computational models of the knee joint can be adopted. Computational models allow to vary and isolate factors affecting knee joint functioning, making them suitable for analysis of the different structures around the knee. However, in order to simulate the effects of ACL interventions, input on the mechanical behavior of the native, intact, ACL is required.

Several studies have analyzed the inherent mechanical properties of the ACL by performing tensile tests in experimental set-ups (Girgis et al., 1975; Arnoczky, 1993; Markolf et al., 1984; Markolf et al., 1990; Markolf et al., 1995). However, these experiments only focused on an active, or tensed ACL in the knee during full extension or
In-situ mechanical behavior and the slack length of the ACL in deep flexion. To properly simulate the ACL function, additional information is needed on the slackness of the ligament, in the intermediate range between full extension and deep flexion. When the slack length of the ACL is underestimated, the ligament becomes active earlier during the flexion movement and close to extension, which affects the kinematic behavior of the joint and increases the joint contact pressure. Overestimating the slackness of the ACL, however, promotes joint instability. Previous studies have shown that the ACL is mainly tensed at low (0-30°) and high (>120°) flexion angles (Markolf et al., 1995). However, it is not known to what extent the ACL becomes slack in the mid-region.

In addition, the ACL is composed of two main fiber bundles, the antero-medial bundle (AMB) and postero-lateral bundle (PLB). Due to this specific morphology, different components of the ligament may be stretched during knee motion (Girgis et al., 1975; Arnoczky, 1983; Zanthop et al., 2006), making the mechanical response of the ACL dependent on the flexion angle of the knee. Hence, the best way to assess the mechanical behavior of the ACL is while it is left in-situ in the knee joint.

The goal of this study was therefore to quantify the slackness and mechanical behavior of the ACL in-situ, at different flexion angles. The mechanical behavior was assessed by measuring the tensile response of the ACL, when varying its length.

2. Materials and Methods

Four fresh-frozen human cadaver knees (72 to 86 years old; 2 males, 2 females; 3 right knees, 1 left knee) were used to measure the tension and slack length of the ACL. Before dissection, the knees were examined in a Magnetic Resonance Imaging (MRI) scan to verify that the ACL was intact. The specimens were thawed 24 hours prior to dissection, after which the knees were prepared for the measurement in a custom knee loading rig (Figure 1). The rig allows for positioning of the knee in each desired position, and was previously used by Barink et al. (Barink et al., 2007). In our study, the position of the tibia was placed in the bracket A, which could be flexed around joint x (indicated as R1 in Figure 1) from full extension (0°) to 150° of flexion, with an accuracy of 1°. The rig also allows for proximal and distal translation by displacing bracket A (T2, Figure 1). The femur was placed in the bracket D, which had four degrees of freedoms: medial-lateral translation (T1), antero-posterior translation (T3), varus-valgus rotation (R3) and internal-external rotation (R2). During the measurements, at each knee flexion angle, we locked all movements of the rig after the knee joint found its self-adjusted position, guided by the soft tissue envelope of the knee.

To fit the knee into the rig, the knees were cut 20cm proximal and 19cm distal to the joint line. Soft tissue within 15cm proximal and 13cm distal to knee joint cavity was left intact. Subsequently, the bones were potted using polymethylmethacrylate (PMMA).
Releasing the ACL

To record the ACL tension curve due to the displacement at a specific knee angle from 0° to 150°, we applied the method of Markolf (Markolf et al., 1990) to isolate the tibial attachment of the ACL. This technique was later applied by Arnold and colleagues in our laboratory to record ACL tension (Arnold et al., 2004). The ACL was released from the tibial attachment by an experienced orthopaedic surgeon (RLD), according to the following procedure.

First, the knee was opened to expose the exact location of the ACL. By using a tibial aiming device (Arthex, Naples, FL, USA), a guide-wire was drilled into the centre of the tibial attachment of the ACL, with the direction and position of the wire parallel to the roof of the notch, with the knee fully extended. The guide-wire position was checked under a fluoroscope in the antero-posterior and lateral projection. After confirming the wire position, a 4.5 mm canulated drill was used over the guide-wire until the subchondral bone under the ACL insertion site was reached. With the guide wire still attached to the bone, a 7 mm canulated screw was driven into the bone over the guide wire, until the screw tip touched the subchondral cortex. Subsequently, a core drill (14 mm outside diameter) was drilled over the guide wire until the subchondral bone plate. The ACL was then further released by removing the surrounding connective tissue. Some additional stitches were used to further ensure adequate fixation of the ACL to the tensiometer. We confirmed complete release of the ACL by pushing and pulling the bone-screw while checking the adequate force response of the ACL.
Mounting the tensiometer

A custom made tensiometer (Figure 2) was used in this study (Arnold et al., 2004), with a force accuracy of +/- 1N. The tensiometer was calibrated prior to each experiment using dead weights. The electric signal of the sensor was connected to an analog-to-digital-circuit (A/D Converter) with a USB interface. The digital data was then further processed using QuickDAQ (Data Translation, Marlboro, USA). The tensiometer frame was mounted firmly to the tibia, while the hook of tensiometer was connected to the ACL. Two sharp pins of the frame were driven into the cortical bone alongside the bone tunnel, to further anchor the tensiometer. The position of the hook was adjusted through a length adjustment screw (part 2 in Figure 2), while the height of the frame could be adjusted to bring the tensiometer in line with the tibial tunnel. The adjustment screw was used to vary the displacement while tensioning and relaxing the ACL tension, with 0.5mm displacement increments.

Testing Procedure

For each knee, the measurement was started by connecting the ACL to the tensiometer and putting the knee in the “base-position”, after which a pre-conditioning sequence was performed. For the base-position, the knee was placed in full extension, after which the ACL tension was set to 10N by adjusting the adjustment screw. This position was based on the study of Arnold et al (Arnold et al., 2004). The pre-conditioning sequence consisted of five consecutive cycles of flexion-extension 0° to 90°.
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After pre-conditioning, we started to measure the tension and slackness of the ACL as a response to the applied displacement and flexion angle. We performed the measurement at flexion angles ranging from 0° to 150°, with 15° increments.

For each flexion angle, the measurement was started by first placing the knee in the base-position (full extension, 10N pre-tension). Next, the knee was placed in the desired flexion angle. After the knee joint found its self-adjusted position, all degrees of freedom of the rig were locked. We then measured the ACL tension which would show either a specific level of ACL tension (1.) or ACL slackness (2. – no reaction force measured). Based on the initial measurement, the measurement was proceeded as follows:

1. In the case that the ACL was under tension, using the adjustment screw, the tension was reduced by increasing the ACL slackness with 0.5mm increments. At each increment, the ACL tension was recorded, until full slackness was reached (tension = 0N). At this point, we would rotate the adjustment screw back to the initial (tensed) position and continue to measure the ACL tension by stretching the ACL with 0.5mm increments. The measurement was stopped when the ACL tension exceeded 20N, to prevent damage occurring to the ACL-tensiometer connection.

2. In case the ACL was initially slack, we would only stretch the ACL by increasing the displacement using the adjustment screw, at 0.5mm increments. Again, at each displacement increment, the ACL tension was recorded, until the ACL tension exceeded 20N.

After measuring the tension-displacement characteristics for a specific flexion angle, the ACL was released again, the knee was placed back in full extension, after which the procedure was repeated for the next flexion angle.

Data Processing

As a verification step, and to provide a comparison with previous studies, first, a standard flexion-tension graph was reproduced. This entailed that the ACL slackness was set to 10N in full extension, after which the flexion angle was changed to 150°, in 15° increments. This measurement should result in the typical U-curve, as seen in previous experiments (Arnold et al., 2004).

Next, the testing procedure as described above was followed. As the experiments were performed at various flexion angles, tension-displacement curves were produced with a horizontal (displacement) offset that depended on the slackness. To determine the slack-length at a specific flexion angle, a cut-off value of 0.5N was taken. The horizontal offset at this tension level was then taken as either the slack length, or the level of pre-tension.

The nominal tension-displacement curves were then determined, by removing the slack-length or pre-tension offset. Theoretically, if the nominal mechanical response of the ACL would be similar in each flexion angle, this would result in nearly identical tension-displacement curves, while the curves would be divergent if the nominal
response would depend on the flexion angle. To quantify this difference we calculated the linearized ACL stiffness for each knee. The linearized stiffness was determined at a tension cut-off value of 15N, and calculated by dividing the tension level (15N) by the nominal displacement (corrected for slackness/pre-tension). We determined the linearized stiffness as a function of knee flexion angle.

Finally, we fitted the acquired data to a ‘single bundle’ ACL model, which can be used for modeling of the ACL in computational models of the human knee.

**Statistical analysis**

Friedman’s non parametric test was performed using SPSS Statistics 19 (SPSS Inc., IL, Chicago) to determine a mean difference of an ACL’s linear stiffness relative to each group of knee flexion angles. In this study, knee flexion angles (ranging from 0° to 150°, with 15° increments) were divided into three different groups i.e. low knee flexion angle (from 0° to 60°), intermediate knee flexion angle (from 75° to 105°) and high knee flexion angle (from 120° to 135°). Due to the limitation of the experimental data samples and the specific (unique) cadaver’s knee properties, subsequently, we performed a Tukey Post Hoc test of General Linear Model Univariate to determine the mean value difference between two groups. The groups of the knee flexion angle (low knee angle, intermediate knee angle, and high knee angle) and the knee cadavers (C545L, C555R, C580R, and C590R) were set as independent variables, whereas the stiffness value was set as a dependent variable. A significance level of alpha < 0.05 and a confidence level of 95% were used for all statistical analysis.

**3. Results**

For three knees we were able to complete the full experimental protocol (0-150°), while in one knee we were unable to apply a flexion angle higher than 135°, due to excessive fat tissue surrounding the knee.

**Standard flexion-tension graph**

All knees displayed the characteristic U-curve of the ACL tension (Figure 3), as previously demonstrated by Arnold et al. (Arnold et al., 2004). The ACL was predominantly tensed in the lower (0° to 60°) and higher (120° to 150°) knee flexion angles, and slack in intermediate flexion angles (75° to 105°).

**Tension-displacement curves**

Figure 4 shows typical tension-displacement curves produced by a single knee (C590R; 78 yrs; female). For each flexion angle, a different tension-displacement curve was produced, providing information on the ACL slack length, and the stiffness response at the various angles. Curves passing through the vertical axis (particularly occurring in lower and higher flexion angles) expressed a pre-tension, while curves shifted to the right (mid-flexion range) were slack in the base position.
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Figure 3. ACL tension (mean value) in an unloaded condition at different knee angles, with a slack limit of 0.5N.

Figure 4. ACL tension-displacement curves of one knee (C590R; 78 yrs; Female). Eleven curves are shown for each individual flexion angle (from 0° to 150° of flexion with 15° increments).

Slackness evaluation

For each knee and each flexion angle, the slack length or level of pre-tension were calculated (Table 1, Figure 5). Slackness was represented as a positive displacement, while pre-tension was represented as a negative displacement. Dependent on the specific knee, the ACL became slack between 30° and 60°, and tensed again between 105° and 150°. Maximum slackness was reached around 75°, with a magnitude of 1.30±0.89mm. The maximum level of pre-tension was reached either at 0° or 150°, with a magnitude of -2.34±0.80mm. An “average” slackness curve was fitted through all data points, based on the four donor knees (Figure 5).
Table 1. Displacement values applied to obtain a slack ACL (0.5 N). A positive value means that the ACL was slack (particularly in the mid-flexion range); whereas a negative value means the ACL was under tension (particularly at the extended and highly flexed regions).

<table>
<thead>
<tr>
<th>Knee</th>
<th>0°</th>
<th>15°</th>
<th>30°</th>
<th>45°</th>
<th>60°</th>
<th>75°</th>
<th>90°</th>
<th>105°</th>
<th>120°</th>
<th>135°</th>
<th>150°</th>
</tr>
</thead>
<tbody>
<tr>
<td>C545L</td>
<td>-1.39</td>
<td>-1.06</td>
<td>0.02</td>
<td>1.32</td>
<td>2.22</td>
<td>1.91</td>
<td>1.04</td>
<td>1.32</td>
<td>1.30</td>
<td>0.38</td>
<td>-1.23</td>
</tr>
<tr>
<td>C555R</td>
<td>-1.94</td>
<td>-1.31</td>
<td>-0.23</td>
<td>0.14</td>
<td>1.42</td>
<td>1.89</td>
<td>1.84</td>
<td>1.15</td>
<td>0.24</td>
<td>-1.44</td>
<td>-3.14</td>
</tr>
<tr>
<td>C580R</td>
<td>-1.65</td>
<td>-1.63</td>
<td>-1.05</td>
<td>-0.59</td>
<td>0.14</td>
<td>0.26</td>
<td>0.59</td>
<td>-0.24</td>
<td>-1.18</td>
<td>-2.83</td>
<td></td>
</tr>
<tr>
<td>C590R</td>
<td>-1.99</td>
<td>-1.26</td>
<td>-0.38</td>
<td>-0.07</td>
<td>0.49</td>
<td>0.46</td>
<td>0.49</td>
<td>0.44</td>
<td>-0.27</td>
<td>-0.44</td>
<td>-1.84</td>
</tr>
</tbody>
</table>

Table 2 showed the ACL linear stiffness at 15N for all knee flexion angles. The statistical analysis only used the ACL stiffness data of knee flexion angle from 0° to 135° as there was a missing value for C580R at 150° of flexion. We compared a mean value of the ACL stiffness among three knee flexion angle groups using the Friedman’s non-parametric tests. The tests showed a significant different of mean value for each knee flexion angle group (p= 0.039). In addition, the multiple pairwise comparison of the Tukey Post Hoc test showed that a significant different between low and intermediate knee flexion angle as well as between high and intermediate knee flexion angle with p=0.008 and p=0.049, respectively.

**Stiffness response**

The offset was then removed in the tension-displacement curves by subtracting the slackness or adding the pre-tension as determined above, causing a horizontal shift of the curves (compare Figure 4 and 6). The nominal curves did not overlap each other, indicating that the stiffness response was not constant for all flexion angles (Figure 6).

Figure 5. Pre-tension and slackness curves for each knee as a function of flexion angle. In the mid-flexion range ligament laxity values up to 2.2 mm were found.
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The linearized stiffness, determined at the 15N cut-off level, ranged from 3.25 N/mm to 10.56 N/mm (Table 2). All knees expressed a similar pattern, with the ACL being stiffer at low and high flexion angles, concurrent with the flexion positions in which the ACL is mostly engaged.

![Fitting curve of the ACL stiffness of one knee (C590R; 78 yrs; Female) at different knee flexion angles](image)

Figure 6. Fitting curve of the ACL stiffness of one knee (C590R; 78 yrs; Female) at different knee flexion angles

Table 2. Linear stiffness of the ACL at 15 N of four knees showing typically higher values close to extension and at larger flexion angles.

<table>
<thead>
<tr>
<th>Knee</th>
<th>Linear stiffness at 15N [N/mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0⁰</td>
</tr>
<tr>
<td>C545L</td>
<td>9.62</td>
</tr>
<tr>
<td>C555R</td>
<td>5.75</td>
</tr>
<tr>
<td>C580R</td>
<td>7.58</td>
</tr>
<tr>
<td>C590R</td>
<td>6.44</td>
</tr>
</tbody>
</table>

4. Discussion

The goal of this study was to quantify the slackness and mechanical response of the ACL in-situ, at different flexion angles. For this purpose, we varied the displacement of the tibial attachment of the ACL while measuring the force response in four fresh-frozen cadaveric knees.

Obviously our study has a number of limitations. The cadaveric knees used in the current study were of a relatively high age (79.0±5.8 years), not resembling the typical young and active patient. Furthermore, we were unable to manipulate the tibial insertion site using the intended screw fixation in the tibial bone-block as performed in the study of Markolf (Markolf et al., 1990), due to poor bone quality. The suture
fixation that was adopted allowed more rotation of the ACL compared to the screw fixation, which potentially could affect the results. However, the bone tunnel provided good visibility of the sutures, and we observed minimal rotational movements of the sutures during the experiments.

Furthermore, the results of the measurements were quite reproducible between knees, indicating a limited effect of the manner of fixation. An actual possible benefit of using sutures instead of a bone plug was that we avoided possible bone-on-bone friction in the bone tunnel. The age of the cadaveric tissue most likely also affected the mechanical response of the ACL tissue, as several studies have demonstrated that age is an important factor in the mechanical properties of ligaments (Hammer et al., 2012; O’Brien et al., 2010; Hashemi et al., 2010; Carroll et al., 1985; Jones et al., 1995), while ACL injuries occur most frequently in younger patients (Markolf et al., 1995).

After release of the ACL the pretension at extension was set at 10N as suggested by Arnold et al. (Arnold et al., 2004). Hence, this initial setting was identical for all knees. This is probably a simplification of reality and pre-tension values are bound to differ between individuals. Currently, as far as we know, there is no way to assess the pre-tension of the ACL under in-situ circumstances. For this reason we decided to standardize it to 10 N and subsequently assess how the ACL’s of four different cadavers respond as a function of flexion angle.

During our experiments we placed the knee at a particular flexion angle, and fixed all degrees of freedom after the knee found its self-adjusted position. This position was guided only by the passive structures surrounding the knee, while in vivo the knee position may be affected by the muscles surrounding the knee. Moreover, while tightening the ACL, the position of the knee was fixed, so that we could measure the actual elongation/shortening of the ACL. In vivo, this ACL tension would also affect the knee position, pulling the tibia in the posterior direction relatively to the femur. These limitations may have affected the mechanical response of the ACL, as we demonstrated that the stiffness depended on the flexion angle, and therefore on the angle of the ACL with respect to the femur and tibia. Allowing for a posterior translation of the tibia, for instance, would have resulted in a steeper orientation of the ACL, with a different mechanical response. Hence, our measurements give an indication of the stiffness response in a passive flexion position, but may be different in an in vivo situation with more degrees of freedom and muscle activation affecting the actual ACL position.

The linearized ACL stiffness as determined in this study is difficult to be compared directly to other studies at all flexion angles. However, the literature does provide information about the average linear stiffness at knee flexion angles 30° and 45°. In our study, the average linear stiffness of the ACL from old donors was 6.36 ± 1.74 N/mm at 30° knee flexion angle and 5.23 ± 0.96 N/mm at 45° knee flexion angle. Those values were different from a previous study of Woo et al. (Woo et al., 1991) and of Noyes and Grood (Noyes and Grood, 1976) who published an ACL linear stiffness of 180 ± 25 N/mm (60-97 years old donors) at 30° knee flexion angle and 129± 39 N/mm (48-86 years old donors) at 45° knee flexion angle, respectively. By analyzing the graphical
load-elongation curve at 30° of flexion in the anatomical orientation of Woo et al. (Woo et al., 1991), we could estimate the linear stiffness at 15N and 20N load which were 53 N/mm and 55.4 N/mm, respectively. Hence, our results show much lower stiffness values which could be partly attributed to the different types of testing and perhaps the age of the knee donors.

Our data came from a direct measurement of tension to the ACL which was caused by the displacement load until the ACL reach the tension around 20 N, whereas the ACL stiffness values from two previous studies were obtained from indirect measurement of the ACL through applying a higher load to the bone (not to the ACL in-situ), with force of 100 N (Woo et al., 1991) and around 600 N (Noyes and Grood, 1976). Another study of Markolf et al. (Markolf et al., 1995) also showed that the ACL force could reach around 200N with external tibial load of 100N. Unfortunately, they did not provide the ACL linear stiffness values as a comparison data. As the ACL is a highly non-linear material, with higher stiffness at higher strain values, it is logical that we found much lower stiffness values than the values produced by Woo et al. (Woo et al., 1991) and by Noyes and Grood (Noyes and Grood, 1976). Hence, the stiffness values found in our study must be judged as the ‘initial linearized stiffness’ of the load-displacement curve. One could argue that we should have used higher loads than our maximal force of 20 N, but pilot studies had shown that loads much higher than that would not allow for the multiple measurements which we wanted to perform in the cadaveric knees.

The current results indicate that the mechanical response of the ACL varies with the flexion angle. The mean ACL stiffness of the intermediate flexion angle group (4.90 N/mm) was significantly different relative to the low flexion angle group (6.07 N/mm) and the high flexion angle group (6.01 N/mm). On the other hand, the mean ACL stiffness of the low flexion angle group was not statistically different relative to the high flexion angle group with significance (p) of 0.990. This suggests that the ACL slackness was not only influenced by the location of the attachment sites relative to the rotating knee axis, but also the knee flexion angle. During the ACL reconstruction, the surgeon should test the ACL stiffness for all knee angle position range. The effective stiffness of the ACL is optimized for positions in which the ACL is mostly active (extension and deep flexion), whereas the slackness of the ACL is optimized during mid-flexion angle. Evidently, this is a result of the double-(or multiple) bundle architecture of the ACL, with the antero-medial bundle being stretched in flexion, and the postero-lateral bundle being stretched in extension (Girgis et al., 1975; Arnoczky, 1983; Zanthop et al., 2006).

Our study may provide further clinical insights, particularly for optimizing the pre-tension and slackness in ACL reconstructions. Although perhaps complex to incorporate in a clinical setting, our results provide guidelines for the amount of slackness in intermediate flexion angles, and for the level of pre-tension in extension and deep flexion. Such a procedure would, however, require a clinically suitable ACL tensiometer, which currently is not standard clinical practice. Using a tensiometer to obtain tension data entails a more invasive approach, and more time at the operating room (Livesay et al., 1995). Moreover, some studies have suggested that the pre-tension applied to ACL
In-situ mechanical behavior and the slack length of the ACL grafts is released soon due to remodeling (Peña et al., 2005) and viscoelasticity (Pioletti et al., 1998) post-operatively.

From a knee modeling perspective, the current results facilitate the fine-tuning of the ACL parameters, such as the slack length (Blankevoort and Huiskes, 1991; Limbert et al., 2004), the flexion-angle dependent stiffness, and the level of pre-tension in certain ranges of flexion. These parameters have been shown to have a significant effect on the tibio-femoral joint kinematics (Dargel et al., 2007; Li et al., 2005). A similar experiment could also be performed to determine the mechanical response of the posterior cruciate and collateral ligaments. The final set of parameters could subsequently be validated against in vivo laxity tests, such as the drawer test, to verify the functional laxity of the knee (Limbert et al., 2004).

We conclude that the slackness and stiffness of ACL are dependent on the flexion angle, in such a way that the mechanical properties are optimized for the region in which the ligament is engaged the most, such as in extension (0-30°) and deep flexion (120-150°). These findings may be of clinical relevance for ACL reconstructive surgery, and are furthermore useful for implementing the mechanical response of the ACL in computational models of the human knee.

Conflict of interest

There are no conflicts of interest

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