A Novel Core Biopsy Technique for Anterior Cruciate Ligament Preserves Ligament Structural Integrity: A Porcine Study

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**Purpose:** The objective of this study was to validate a new technique to safely obtain core biopsy specimens of the anterior cruciate ligament (ACL) without jeopardizing the ACL’s biomechanical properties. **Methods:** Eleven pairs of fresh porcine femur-ACL-tibia complexes were tested in a loading frame. The ACL of one knee was biopsied using a spring-loaded core biopsy device, whereas the contralateral ACL was tested as the control. Biomechanical properties of the biopsied and control ACLs were compared. **Results:** The ultimate load to failure was 1,202 N ± 171.1 N and 1,193 N ± 228.7 N (P = .8984) for biopsied and non-biopsied ACLs, respectively. No significant differences were noted for maximal elongation at failure, maximal strain, absorbed energy, and stiffness between biopsied and non-biopsied ACLs. **Conclusions:** The results of this study indicate that a new ACL core biopsy technique can be performed while preserving the ligament’s structural integrity. **Clinical Relevance:** The presented core biopsy technique could be regarded as a dedicated tool to elucidate the poorly understood (patho)biological processes occurring in both the native and reconstructed ACLs.

According to the literature, 2.6% to 7.7% of anterior cruciate ligament (ACL)—reconstructed patients have graft failure develop with recurrent instability and will require revision ACL reconstruction. A recent report of the Multicenter ACL Revision Study (MARS) study group identified that the cause of ACL graft failure can be traumatic (32%), technical (24%), biological (7%), a combination (37%), infection (<1%), and not known (<1%). Unlike reasons for technical failure of ACL reconstruction, the underlying mechanisms leading to a biological graft failure (i.e., non-traumatized though nonfunctional graft) remain largely unclear. The diagnosis of biological graft failure is therefore predominantly based on exclusion of other reasons for failure, thus likely creating an underestimation of this enigmatic mode of graft failure.

Animal studies have shown that after a period of time, the implanted tendon grafts undergo a process of several phases to become ligamentous “ACL-like” structures. The process of “ligamentization” of tendon grafts is indeed believed to consist of an initial phase of necrosis followed by revascularization, cellular repopulation, and remodeling. Today, however, there is growing evidence that the results of animal studies cannot be directly applied to human graft biology. According to one of our articles, humans and pigs are indeed different in a biological way, for example, in the timeline of ligamentization. However, there is good evidence that the ex vivo anatomy and biomechanics are quite similar. For this study, only time 0 ex vivo biomechanical aspects were of interest.

All current knowledge on human ACL graft biology is derived from superficial biopsy specimens obtained with a basket forceps during arthroscopy. This means that all available information on the biology of the human reconstructed ACL is based on biopsy specimens of a peripheral portion of the graft. The question remains whether such biopsy specimens can be representative of the entire 3-dimensional graft structure. In this view, we previously concluded that the development of a core biopsy technique is a prerequisite to better understand the biology of the healing ACL graft in the human knee.
Ideally, the technique must meet following requirements: safe, reproducible, minimally invasive, and providing representative tissue samples with dimensions enabling histologic analysis. Given its widespread clinical use in other medical disciplines, a spring-loaded needle core biopsy device was chosen as a possible candidate to fulfill all those requirements. For safety evaluation with regard to the structural integrity of the ACL after biopsy, a study was set up to compare the biomechanical properties of a biopsied ACL with the control native ACL in the contralateral knee.

The purpose of this study was to validate a new technique to safely obtain core biopsy specimens of the ACL without jeopardizing the ACL’s biomechanical properties. We hypothesized that there would be no difference in biomechanical properties between the biopsied and non-biopsied ACLs.

Methods

Prior sample size calculation showed that 22 paired (11 × 2) fresh cadaveric porcine knees were needed to obtain a power of 0.8. Pigs were chosen because the physical dimensions and biomechanical properties of the porcine ACL have been shown to closely mimic those of the human ACL, with the advantage of being easily available in a fresh state. The pigs were killed at a mean age of 180 days, according to the rules for human consumption regulated by Belgian law. The first author was present during packaging of the specimens to guarantee the correct pairing of the knees from the same cadaver. The paired knees were then sealed in a plastic bag and stored in the refrigerator at 3°C, with the experiments being performed the day after slaughter.

At the start of the experiments, both the femur and tibia were cut with an oscillating saw at a level 15 cm away from the joint line. The knees were then dissected and stripped of all surrounding soft tissues except for the ACL, which was carefully isolated. The femur-ACL-tibia (FAT) complex was kept moist with saline solution spray before and during testing. Knees with a gross deformity or the slightest visible damage to the ACL were excluded. Ethical approval for this study was granted through our university’s committee for medical ethics.

Testing Protocol

The FAT complex was subsequently potted firmly in custom-made metal cups by use of a resin consisting of 2-hydroxypropyl methacrylate (VersoCit-2; Struers A/S, Ballerup, Denmark). To avoid slippage of the femur or tibia, an additional bolt was placed through a custom-made hole in the cups. Both metal cups were then mounted onto the loading frame (MTS BIONIX 858 Axial Torsional test system; MTS Systems, Eden Prairie, MN) so that the FAT complex was placed on the loading frame with the knee at 30° of flexion, the so-called tibial orientation described by Woo et al. (Fig 1). Preloading to 5 N was performed to allow a method of standardization of the specimens before testing. The dimensions of the ACL (length and anteroposterior and mediolateral diameter at the midsubstance) were measured 3 times with a Vernier caliper (± 0.05 mm). The mean anteroposterior and mediolateral width and length of the ACL were 4.96 mm, 6.77 mm, and 39.93 mm, respectively. Paired specimens were randomized by the toss of a coin to either undergo biopsy or act as a control. All experiments were performed at room temperature.

In the biopsy group, a single core biopsy was obtained with the use of the Monopry disposable biopsy instrument (Bard Peripheral Vascular, Tempe, AZ). The Monopry instrument is a fully automated biopsy device that automatically triggers a rapid-firing side-notch core biopsy needle. It uses a 2-stage biopsy action: a spring action thrusts the inner trocar forward, followed almost instantaneously by a similar forward thrust of the outer cutting cannula. Thus the tissue specimen is trapped in the side notch of the trocar when the cutting cannula is advanced. For the aim of this study, the Monopry 121610 instrument measuring 16 gauge × 10 cm long

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**Fig 1.** FAT complex on loading frame. The ACL was aligned in the direction of elongation.
with a penetration depth of 22 mm and a sample notch length of 17 mm was used. Biopsy specimens were systematically obtained from the mid-portion of the ACL while the trocar rested against the cartilage of the medial femoral condyle to induce some reproducibility (Figs 2-4).

Finally, an uniaxial tensional load on the FAT complex was generated by the loading frame by displacing the femur at a rate of 0.33 mm/s, whereas the tibia was held stationary, until a point well beyond the ACL had snapped. The ACL was aligned in the direction of elongation according to the recommendations of Woo et al.13 and Paschos et al.14

The load cell was connected to a computer, recording the load-displacement data at 100 Hz. The highest point of the force-displacement curve was considered the ultimate failure point. Strain was calculated as the ratio of displacement to the initial length of the ACL and stress as the ratio of load to the initial cross-sectional area. Energy absorption (in newton millimeters) was obtained by taking the area under the force-displacement curve up to the failure point (Fig 5).

Stiffness (in newtons per millimeters) was calculated from the slopes of the linear portion of the force-displacement curve obtained from testing to failure. Accordingly, the Young modulus (in megapascals) was obtained from the stress-strain curve.

**Statistical Analysis**

A sample size calculation was performed to detect a change in maximum load based on a 2-sided paired t test with \( \alpha \) equal to .05. Assuming an SD equal to 250 N\(^1\) and a correlation of 0.5 between paired data, a total of 11 paired specimens were needed to obtain a power of 0.8 to detect a difference in ultimate failure load of 235 N. Data are expressed as mean ± standard deviation. A paired Wilcoxon signed rank test was used to compare the measurements between control and biopsy groups. Analyses have been performed with SAS software (SAS System for Windows, version 9.2; SAS Institute, Cary, NC).
Results

Seven variables were analyzed and compared between biopsied and control knees: ultimate load to failure, elongation at failure, maximum strain, maximum stress, energy absorption, Young modulus, and stiffness. The values for these parameters are summarized in Table 1 and reported as mean ± standard deviation.

After statistical analysis, no significant difference could be found between the control group and the biopsy group for any of the tested variables. Even more importantly, the mean differences for ultimate load (9 N), elongation (0.15 mm), stress (3.64 MPa), strain (0.005), stiffness (3.8 N/mm), Young modulus (2.3 MPa), and energy absorption (243 Nmm) between the 2 conditions seemed of no clinical relevance at all. The difference in percentage is shown in Fig 6.

Discussion

The purpose of this study was to document the safety of a new core biopsy technique for obtaining central tissue samples of the ACL. The results confirm that using our technique, the mechanical properties of the native ACL are unaffected.

Given this result, the proposed technique enables the safe procurement of a piece of tissue obtained from the core an ACL while avoiding mechanical damage. Given this biopsy device’s widespread use in other surgical disciplines, the tissue sample size obtained by this device is definitely large enough for histologic investigation.10,11 In this view, this study might offer a tool that is able to safely deliver tissue samples from the very core of a reconstructed human ACL to study its ligamentization process without causing damage to the graft’s biomechanical properties.

Ntoulia et al.16 investigated the graft revascularization process in humans after ACL reconstruction with magnetic resonance imaging (MRI). Although MRI might be a noninvasive procedure to investigate the revascularization phase of the ligamentization process, there is no proven correlation between these MRI findings and histologic properties. Furthermore, revascularization forms only one aspect in the healing process of an ACL graft.

Core biopsy specimens as a diagnostic tool in biological failure of the graft or mucoid degeneration could be used in the individual patient. Their potential applications in clinical practice are therefore multifold.

Ménétrey et al.5 concluded that the pathologic entity of biological graft failure, though accounting for at least 7% of the failures after ACL reconstruction,4 is still poorly understood. In this field the implementation of this new, minimally invasive biopsy technique in suspected cases might show important pathobiological clues surrounding this enigmatic biological graft failure, providing a direct diagnosis of this entity rather than a diagnosis by exclusion and allowing possible preventive or therapeutic measures.

In the same view, remnant-preserving techniques for ACL reconstruction have recently gained interest with their assumed benefit related to an improved or accelerated ligamentization process.17 It has been suggested that the revascularization phase of the ACL graft might start earlier and that the proprioceptive function of the ligament could be better preserved with this technique. Löcherbach et al.18 for example, concluded that this technique could form a possible advantage for ACL reconstruction, although currently, no reliable method exists to investigate graft revascularization and healing after ACL surgery. Moreover, in this field the possibility to obtain safe and representative core biopsy specimens of the ACL graft has the potential to show the added value of such a remnant-preserving technique at the biological level.

Mucoid degeneration of the human ACL is still an intriguing clinical entity that is more frequently encountered than previously thought.19 Typical clinical findings are posterior knee pain and restriction of knee flexion and extension. The diagnosis is made based on clinical presence and MRI. Current treatment with arthroscopic partial excision has been shown to be effective and safe, although postoperative laxity remains a subject of debate. This pathology is histologically characterized by mucoid degeneration of the ACL tissue with accumulation of glycosaminoglycans between the collagen fibrils.20 Makino et al.21 found that mucoid degeneration could be histologically confirmed in only 6 of 10 patients with clinical and MRI criteria for mucoid degeneration. Therefore a more

Table 1. Means and Standard Deviations for All Biomechanical Parameters in Biopsy and Control Groups

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Maximum Load (N)</th>
<th>Elongation (mm)</th>
<th>Stress (MPa)</th>
<th>Strain (Ratio)</th>
<th>Stiffness (N/mm)</th>
<th>Modulus (MPa)</th>
<th>Energy Absorption (Nmm)</th>
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<tr>
<td>Biopsy group</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1,202</td>
<td>10.66</td>
<td>44.81</td>
<td>0.271</td>
<td>185.5</td>
<td>293.5</td>
<td>7,088</td>
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<tr>
<td>SD</td>
<td>171.1</td>
<td>3.33</td>
<td>9.5</td>
<td>0.095</td>
<td>46.51</td>
<td>103</td>
<td>2,665</td>
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<tr>
<td>Control group</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1,193</td>
<td>10.51</td>
<td>48.45</td>
<td>0.266</td>
<td>181.7</td>
<td>295.8</td>
<td>6,845</td>
</tr>
<tr>
<td>SD</td>
<td>228.7</td>
<td>3.38</td>
<td>10.87</td>
<td>0.081</td>
<td>35.87</td>
<td>86.65</td>
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<tr>
<td>P value</td>
<td>.8984</td>
<td>.8984</td>
<td>.2402</td>
<td>.9658</td>
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efficient method to diagnose this pathologic entity more accurately would be useful. In this way the presented core biopsy technique could earn its place in mucoid degeneration as a diagnostic tool but also for further research into the exact histologic processes and causative mechanisms.

The quest to develop an off-the-shelf synthetic graft for ACL reconstruction has delivered variable results. The ideal graft is an ACL scaffold that meets the functional mechanical demands immediately followed by a gradual degradation while host tissue grows in.

Finally, attempts to enhance biological healing processes have gained much interest in the orthopaedic community. The use of platelet-rich plasma and vascular endothelial growth factor has been proposed to improve the biological healing of ACL grafts. However, their effect on clinical outcome remains unclear. Whereas the biology of ACL graft healing remains a hot topic, the results of this study could offer a dedicated tool to gain a better understanding of the process of biological graft failure after ACL reconstruction, the enigmatic entity of mucoid degeneration of a native ACL, and the biological effects of growth factor application (e.g., platelet-rich plasma) or remnant-preserving ACL reconstruction techniques on graft healing.

Limitations

Our study has some limitations. First, an animal model was used for our analyses. Even though pigs have been shown to be a good animal model for studies with ACLs, the results may not automatically apply to humans.

Second, the biopsy would have ideally been performed in living animals. A healing effect of the biopsy specimen could occur in vivo with less damage to the ACL, and therefore we require further assurance of its safety in use.

Finally, the biopsy specimens in this study were obtained from native porcine ACLs, whereas biopsy specimens from in situ ACL grafts undergoing the process of ligamentization possibly would mimic the mechanical properties of the human healing graft even better. However, this study design would entail an extremely complex setup involving porcine ACL reconstruction procedures and subsequent slaughtering and biomechanical testing weeks after the operation.

Conclusions

The results of this study indicate that a new ACL core biopsy technique can be performed while preserving the ligament’s structural integrity.

References


