Preservation of Remnant Tissue Improves Knee Stability and Graft Healing after Anterior Cruciate Ligament Reconstruction in Sheep

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Introduction: It is well known that the ACL-injured knee frequently has a ligament remnant tissue [1], in which mechanoreceptors and free neural ends are found [2-5]. Therefore, theoretically, there is a strong possibility that preservation of the ACL remnant tissue may be able to restore proprioceptive function of the graft after ACL reconstruction. In addition, preservation of the ACL remnant tissue may enhance the revascularization and cellular proliferation of the graft after ACL reconstruction, because the ACL remnant tissue has good subsynovial and intrafascicular vascularity [5-6]. Therefore, several investigators have developed ACL reconstruction with preservation of the remnant tissue [7-10]. However, no basic studies have shown any biological and biomechanical evidence about the utility of the remnant ACL tissue preservation in ACL reconstruction using a large animal model as of yet. Therefore, we have conducted a controlled laboratory study with sheep to clarify the biomechanical and biological effect of the remnant tissue preservation in ACL reconstruction. We have hypothesized that graft coverage by the remnant tissue in ACL reconstruction may significantly improve the anterior-posterior (A-P) knee stability and the recovery of mechanoreceptors. The purpose of this study was to test this hypothesis.

Methods: Forty mature sheep (Suffolk) were randomly divided into 2 groups of 20 animals each. In each animal, the right knee underwent ACL reconstruction using the doubled semitendinosus tendon autograft under general anesthesia according to our previous studies [11-13]. In Group I, the ACL ligament tissue was completely resected before ACL reconstruction. In Group II, the ACL tissue was transected with a scalpel at the mid-substance. Then, the graft was introduced through the tibial tunnel and the ligament tissue into the femoral tunnel. In each group, the graft was placed in bone tunnels, and fixed with an Endobutton (S&N) and a post-screw at 60° of knee flexion under the initial tension of 40 N [13]. Each 10 animals were sacrificed at 4 and 12 weeks after surgery, in Groups I and II, respectively. In each group, 14 knees were used for biomechanical evaluations, and the remaining 6 knees were used for histological and immunohistochemical evaluations. In biomechanical evaluation, the A-P translation of the tibia to the femur was measured using a 3 degree-of-freedom fixture under +/-50N A-P forces at 30°, 60°, and 90° of knee flexion. The cross-sectional area (CSA) of the whole graft was measured with a non-contact optical method using a video dimension analyzer [12]. The structural properties of the femur-graft-tibia complex were determined in tensile testing at a cross-head speed of 50 mm/min. In histological observation, the reconstructed ACL graft was stained with hematoxylin and eosin. The
samples were subjected to immunohistochemical analysis with monodonal antibodies against S100 protein and α smooth muscle actin (SMA). Statistical analyses were made using the Student’s t test. Significant level was set at p=0.05.

**Results:** Concerning the tissue dimension, there was no significant difference between the two groups at 4 and 12 weeks after surgery in the ACL graft length. The CSA of the ACL graft was significantly thicker in Group II (p=0.0143) than in Group I at 12 weeks, while there was no significant difference between the groups at 4 weeks. Regarding the A-P translation at 12 weeks (Fig 1), it did indicate significant differences between the groups at 30°, 60°, and 90° of knee flexion (p=0.0157, p=0.002, and p=0.0196, respectively). At 12 weeks, the mean initial stiffness and terminal stiffness of the load-displacement hysteresis loop were 0.53 N/mm and 23.2 N/mm, and 1.35 N/mm and 22.3 N/mm in Groups I and II at 60° of knee flexion, respectively. The initial stiffness was significantly greater in Group II (p=0.032) than in Group I, while there was no significant difference in the terminal stiffness between the groups (Fig 2).

In tensile testing, all specimens appeared as being pulled out from the tibial tunnel in each group at 4 weeks, while all specimens failed at the mid-substance in each group at 12 weeks. The mean maximum load was 204.0 N, and 155.0 N, the stiffness was 83.4 N/mm, and 60.6 N/mm, and the elongation at failure was 7.3 mm, and 4.5 mm, in Groups I and II, respectively, at 4 weeks, while the mean maximum load was 300.7 N, and 393.9 N, the stiffness was 91.9 N/mm, and 95.6 N/mm, and the elongation at failure was 6.3 mm, and 5.4 mm, in Groups I and II at 12 weeks. There were no significant differences in each parameter between the groups at 4 and 12 weeks after surgery (Fig 3).

Histologically, in the periphery of the graft, a thin synovial tissue was covered around the graft with flat epithelial cells in Group I at 4 and 12 weeks. In Group II at 12 weeks, a thick synovial tissue was covered around the graft. The continuity between the remnant tissue and the graft was observed in Group II at 12 weeks. Collagen bundles were almost longitudinally oriented, and cells with an oval or rod-like nucleus infiltrated into the graft. However, the core portion of the graft remained necrotic in Groups I and II at 12 weeks. Morphologically, mechanoreceptors and proprioceptive fibers were found in all graft of Group II at 4 and 12 weeks (Fig 4), while these were not identified in Group I. The mean number of mechanoreceptors in Group II was significantly greater at 12 weeks than at 4 weeks (p=0.016). αSMA positive blood vessels were found in the graft of Groups I and II at 4 weeks (Fig 5). Vessels were stained thicker in Group II than in Group I without irregularity.

**Discussion:** This study clearly demonstrated that a remnant tissue coverage significantly improved the A-P translation, the initial stiffness of the load-displacement hysteresis loop, and the recovery of mechanoreceptors at 12 weeks after surgery. These results implied that the sufficient remnant tissue coverage significantly enhances healing of the tendon graft after ACL reconstruction. We consider that the sufficient fibrous tissue coverage of the grafts may reduce elongation or failure of the grafts in the graft-remodeling phase, resulting in better knee stability. As to clinical relevance, preservation of the ACL remnant tissues may be of potential benefit during ACL reconstruction, as some re-innervation and the recovery of proprioceptive potential may be possible, thus improving clinical outcomes.

**Significance:** This study clearly demonstrated that a remnant tissue coverage significantly improved the anterior-posterior translation and the recovery of mechanoreceptors after ACL reconstruction at 12 weeks after surgery.
Fig 1. The anterior-posterior translation of the tibia to femur at 12W

Fig 2A. The initial stiffness at 12W  
Fig 2B. The terminal stiffness at 12W

Fig 3. The structural properties of the femur-graft-tibia complex at 12W
**Fig 4.** Histological observation of the ACL graft in Group II. A: S100 positive Ruffini corpuscle (x40). B: S100 positive Pacini corpuscle (x40). C: S100 positive Golgi tendon organ (x20).

**Fig 5.** Histological observation of the ACL graft. A: αSMA positive blood vessel of the graft in Group I (x10). B: αSMA positive blood vessel of the graft in Group II (x10).