Effects of Remnant Tissue Preservation on Biomechanical Properties of The Tendon Graft 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 sub synovial and intrafascicular vascularity [5]. Therefore, several investigators have developed ACL reconstruction with preservation of the remnant tissue [6-9]. However, no basic studies have shown any biological and biomechanical evidence about the utility of the remnant ACL tissue preservation in ACL reconstruction as of yet. Therefore, we have conducted a controlled laboratory study with sheep to clarify the biomechanical 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. The purpose of this study was to test this hypothesis.

Methods: Twenty mature sheep (Suffolk) were randomly divided into 2 groups of 10 animals each. In each animal, the right knee underwent ACL reconstruction using the doubled semitendinosus tendon graft under general anesthesia according to our previous studies [10-12]. 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 [12]. All animals were sacrificed at 12 weeks after surgery. In each group, 7 out of the 10 sheep were used for biomechanical evaluation, and the remaining 3 were used for histological and immunohistochemical observation. In biomechanical evaluation, the A-P translation of the tibia to the femur was measured using a 3-DOF 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 an optical method using video dimension analyzer [11]. 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 haematoxylin and eosin. The samples were subjected to immunohistochemical analysis with monoclonal antibodies against S100 protein. Cells infiltrating in the core portion of the graft were counted in 3 longitudinal sections, and the mean cell density was determined. Statistical analyses were made using the Student’s t test. Significant level was set at p=0.05.

Results: Concerning the tissue dimension (Fig 1), there was no significant difference between the two groups in the ACL graft length. The CSA of the ACL graft was significantly thicker in Group II (p=0.0143) than in Group I. Regarding the A-P translation (Fig 2), 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). In tensile testing, all specimens failed at the mid substance in each group. 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, respectively. There were no significant differences in each parameter between the groups (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. In group 2, a thick synovial tissue was covered around the graft. The continuity between the remnant tissue and the graft was observed in Group II (Fig 4A). 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. Concerning the cell density, there was no significant differences between Groups I (921 cells/mm\(^2\)) and II (1131 cells/mm\(^2\)). Morphologically, mechanoreceptors and proprioceptive fibres were found in all graft of Group II, while these were not identified in Group I.

Discussion: This study clearly demonstrated that a remnant tissue coverage significantly improved the A-P translation after ACL reconstruction at 12 weeks after surgery. This result 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. There are some limitations in this study. The first one is that we performed only the standard histological examinations. We should conduct a molecular biological study in the near future to find objective differences in the cell function and the graft matrix between the groups. The second one is that there were no significant differences concerning the structural properties of the graft. As to clinical relevance, preservation of the ACL remnant tissues may be of potential benefit during ACL reconstruction, as some re-
innervation and recovery of proprioceptive potential may be possible, thus improving clinical outcomes.

**Significance:** This study demonstrated that a remnant tissue coverage significantly improved the A-P translation after ACL reconstruction at 12 weeks after surgery.

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![Graph 1](image1.jpg)  
**Fig 1.** The tissue dimension of the ACL graft.

![Graph 2](image2.jpg)  
**Fig 2.** The anterior-posterior translation of the tibia to the femur.
Fig 3. The structural properties of the femur-graft-tibia complex.

Fig 4. Histological observation of the ACL graft in Group II (A: HE stain (x10), B: Pacinian-like mechanoreceptors (x50), C: Nerve endings S100 positive (x50)).

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