Anterior cruciate ligament remnant-preserving and re-tensioning reconstruction: a biomechanical comparison study of three different re-tensioning methods in a porcine model

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Abstract

Background: With the developments in the arthroscopic technique, anterior cruciate ligament (ACL) remnant-preserving reconstruction is gradually gaining attention with respect to improving proprioception and enhancing early revascularization of the graft. To evaluate the mechanical pull-out strength of three different methods for remnant-preserving and re-tensioning reconstruction during ACL reconstruction.

Methods: Twenty-seven fresh knees from mature pigs were used in this study. Each knee was dissected to isolate the femoral attachment of ACL and cut the attachment. An MTS tensile testing machine with dual-screw fixation clamp with 30° flexion angle was used. The 27 specimens were tested after applying re-tensioning sutures with No. 0 polydioxanone (PDS), using the single stitch (n = 9), loop stitch (n = 9), and triple stitch (n = 9) methods. We measured the mode of failure, defined as (1) ligament failure (longitudinal splitting of the remnant ACL) or (2) suture failure (tearing of the PDS stitch); load-to-failure strength; and stiffness for the three methods. Kruskal-Wallis test and Mann-Whitney U-test were used to compare the variance of load-to-failure strength and stiffness among the three groups.

Results: Ligament failure occurred in all cases in the single stitch group and in all but one case in the triple stitch group. Suture failure occurred in all cases in the loop stitch group and in one case in the triple stitch group. The load-to-failure strength was significantly higher with loop stitch (91.52 ± 8.19 N) and triple stitch (111.1 ± 18.15 N) than with single stitch (43.79 ± 11.54 N) (p = 0.002). With respect to stiffness, triple stitch (2.50 ± 0.37 N/mm) yielded significantly higher stiffness than the other methods (p = 0.001).

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Conclusions: The results suggested that loop stitch or triple stitch would be a better option for increasing the mechanical strength when applying remnant-preserving and re-tensioning reconstruction during ACL reconstruction.

Keywords: ACL, Remnant-preserving, Re-tensioning, Biomechanical

Background
Despite the recent successful outcomes of anterior cruciate ligament (ACL) reconstruction, the failure rate is still 8–25% [1, 2]. For successful ACL reconstruction, various factors, including graft placement with firm fixation, incorporation, revascularization, and ligamentization, should be considered [2–4]. In addition to stability, good proprioceptive function is important [1, 4, 5]. Histologic studies of ACL remnant tissue have revealed the presence of mechanoreceptors and biological healing potential owing to vascular support by the synovial sheath [2, 4, 6]. With the developments in the arthroscopic technique, ACL remnant-preserving reconstruction is gradually gaining attention with respect to improving proprioception and enhancing early revascularization of the graft [1, 2, 4, 7].

The method of preserving the remnant fibers greatly varies from study to study, as follows: (1) merely leaving the tibial portion of the ACL stump [4, 8, 9], (2) selective-bundle augmentation in the presence of an abundant remnant bridging the femur and tibia [10, 11], and (3) femoral avulsion repair and augmentation with graft [1, 7, 12, 13]. However, preservation of the remnant ACL stump might lead to cyclops lesion formation, graft impingement, or incorrect tibial tunnel placement [1, 14]. The clinical results of the remnant-preserving method are still debated [2, 8].

Mechanoreceptors for proprioception can be stimulated by length changes, and the rate of changes in tension. [1, 15–18]. Furthermore, the strength of the remnant tissue in the early phase after reconstruction may be beneficial for rehabilitation and early incorporation [19]. Nagai et al. [20] reported that ACL remnants partially contributed to anterior–posterior stability. For these reasons, it would be better to provide a re-tensioning method to create a mechanically stable environment as possible [19].

Several methods of remnant-preserving and re-tensioning have been introduced, including (1) single stitch, (2) loop stitch, and (3) triple stitch [1, 7, 12, 13]. However, to our knowledge, there are no data on comparative outcomes of the ACL remnant-preserving and re-tensioning reconstruction technique in terms of mechanical strength. The aim of this study was to evaluate the time-zero mechanical pull-out strength with three different re-tensioning methods used in ACL reconstruction. We hypothesized that using the triple stitch method, rather than the simple stitch or loop stitch method, would result in better biomechanical outcomes.

Methods
Specimen preparation for the acute ACL complete femoral detachment model
Twenty-one fresh frozen knees from mature pigs (body weight, 127 ± 11.6 kg) were used in this study (Cellumed, Seoul, Republic of Korea). Each knee was dissected to remove the skin, muscle, collateral ligament, posterior cruciate ligament, posterior cruciate ligament, medial and lateral meniscus, and patella. With any attachment of soft tissue between femur and tibia, after isolating the femoral attachment of ACL, we cut the ACL femoral attachment. We attempted to reproduce the ACL injury pattern (Type I or Type II, suggested by Sherman et al. [21]) as much as possible by cleanly transecting the ACL by three or four times from its femoral insertion site using a No. 11 blade (Fig. 1A). The remainder of the ACL, including the mid-substance and the tibial insertion site, was kept intact. The tibia bone was cut with an oscillating saw 7 cm below the knee joint line. All specimens were wrapped in gauze soaked in saline solution and stored at -4 °C until testing. Before the test day, the specimens were thawed for 6 h at 4 °C [7].

Repair methods
To set the same condition, one type of suture material was used (No. 0 polydioxanone [PDS® II]). Single stitch was made by passing one PDS No. 0 from the medial to lateral direction on the mid-substance of the ACL remnant (Fig. 1B) [13]. Loop stitch was formed by passing one suture loop through the mid-portion of the remnant, and the free ends of the suture were retrieved through the loop [12] (Fig. 1C). Triple stitches were formed sequentially in the medial to lateral, anterior to posterior, and medial to lateral directions on the mid-substance portion of the remnant using three PDS No. 0 (Fig. 1D) [1].

Load-to-failure test
The tensile test was performed on a tensile tester (858 Mini Bionix II; MTS Systems, Eden Prairie, MN). The tibia was fixed on a cylinder with dual-screw (6.0 mm)
and nut fixation. Thereafter, the cylinder on which the tibia was fixed was mounted on a custom-made adjusting device. The PDS suture was also tied and mounted on the custom-made device. The specimen was clamped in the testing fixture according to the instructions and tested at 30° of flexion angle [7]. The clamped sample was adjusted so that the direction of the axial load was aligned with the long axis of the grafted tendon (Fig. 2).

A preload of 2 N was applied to the fixed specimens [7]. The tensile test was started at a crosshead speed of 5 mm/min [22]. The force resolution of the tensile tester was 0.01 N. A load-displacement curve was plotted. The ultimate load-to-failure and stiffness of the specimens were calculated. Stiffness was determined during load-to-failure testing from the maximal endpoint of the linear region of the load-displacement plot, and the relation between ultimate load and displacement was evaluated [7, 23]. The failure mode was determined through visual inspection and defined as (1) ligament failure (splitting of the remnant ACL) or (2) suture failure (tearing of the PDS stitch). Two experimental observers confirmed the failure mode [24, 25].

**Statistical analysis**

Kruskal-Wallis test was used to compare the load-to-failure and stiffness among the three groups. The level of statistical significance was set at $p < 0.05$. The Mann-Whitney U-test was used to compare between-group differences assessed using Bonferroni’s adjustment for multiple testing ($p < 0.05/3$). On the basis of the data obtained in the first six specimens (2 for each group, total of 6), a sample size calculation ($\alpha = 0.05$, $\beta = 0.2$) was
conducted in terms of the mean and standard deviation of load-to-failure using G power 3.1 [26, 27]. A sample size of seven specimens in each group was calculated as the minimum requirement to ensure 80% power for detecting differences in load-to-failure. Finally, a total of 21 knees evaluated. Statistical analysis was performed using SPSS software for Windows (version 25.0; SPSS, Chicago, IL).

**Results**

**Mode of failure**

Ligament failure occurred in all cases in the single stitch group and in all but one case in the triple stitch group. Suture failure occurred in all cases in the loop stitch group and in one case in the triple stitch group. In the single stitch group, the load was dramatically decreased as mid-substance ligament failure occurred after reaching the ultimate load. As a result, a ligament longitudinal splitting failure pattern was observed in all cases (Fig. 3A). In the loop stitch group, failure occurred when the PDS suture was broken at approximately 90 N load (Fig. 3B). There was no injury in the ligament. In the triple stitch group, as the stitches failed one after another after reaching the ultimate failure, the load was stepwise decreased and the final failure occurred, resulting in the ACL fiber being split into three pieces (Fig. 3C).

**Load-to-failure and stiffness**

The load-to-failure strength was significantly higher with loop stitch (91.52 ± 8.19 N) or triple stitch (111.1 ± 18.15 N) than with single stitch (43.79 ± 11.54 N) (p = 0.002) (Fig. 4A). However, there was no significant difference between loop stitch and triple stitch (p = 0.18).

With respect to stiffness, there was a significant difference in the means of the three methods based on the Kruskal-Wallis test (p < 0.001). The Mann-Whitney U-test was again performed for pairwise comparisons. The triple stitch group had the highest stiffness (2.50 ± 0.37 N/mm), closely followed by the loop stitch group (2.13 ± 0.12 N/mm) and finally by the single stitch group (1.24 ± 0.22 N/mm) (Fig. 4B). The triple stitch method yielded significantly higher stiffness than the loop stitch method (p = 0.001) and the single stitch method (p < 0.001).

**Discussion**

The most important finding of this study was that the loop stitch and triple stitch methods provided higher time-zero load-to-failure strength. Moreover, the triple stitch method yielded the highest stiffness among the three re-tensioning methods. Thus, our hypothesis was proved to be correct. The failure pattern was observed to differ according to the repair method. As a result, the single and triple stitch groups had longitudinal tissue splitting patterns, and all cases in the loop stitch group failed at approximately 90 N, which is similar to the ultimate load-to-failure strength of PDS No. 0 [28]. The single and triple stitch methods have the advantage of being technically easy and simple to perform; however, if excessive force is applied during re-tensioning, there is a risk of additional damage to the ACL remnant fibers. Further, the triple stitch method may be difficult to apply depending on the remnant tissue quality and status [29].

With respect to the loop stitch method, better wrapping and coverage around the graft can be achieved [12], but a single stitch could not yield sufficient strength. Therefore, adding a single simple stitch to the loop stitch could result in greater mechanical strength. The disadvantage of the loop stitch method is that it is technically...
demanding and time consuming through the arthroscopy procedure, and additional equipment (Knee Scorpion; Arthrex, Naples, FL) would be required to make it more convenient to use.

Theoretically, as in posterior cruciate ligament reconstruction, augmentation with remnant tissue might be helpful for biological healing in ACL reconstruction [1]. Moreover, remnant tissue helps in revascularizing the graft, incorporating the graft [30], preserving proprioception [15, 19], reducing tibial tunnel widening [8], reducing synovial fluid leakage [13], and mechanically protecting the graft from being scratched by the intercondylar notch [13]. The preserved proprioceptive nerve fibers would reinnervate the reconstructed ACL [4]. On the other hand, some studies showed no difference between “remnant-preserving” and “conventional single-bundle” ACL reconstruction [8, 31]. However, the advantages of remnant preservation are difficult to reveal

Fig. 3 Mode of failure and representative load-to-failure graph for each method: (a) single stitch, (b) loop stitch, and (c) triple stitch *N: newtons, S: second

Fig. 4 Box plot for mean load-to-failure strength and stiffness for each method (7 legs for each method). a Single stitch yielded significantly lower load-to-failure strength (#, p = 0.002). b Single stitch also yielded significantly lower stiffness (#, p < 0.001), whereas triple stitch yielded the highest stiffness among the three repair methods (☆, p = 0.001). ∗N: newton, error bar: range ‡statistical analysis: Kruskal-Wallis test with Bonferroni post hoc analysis
using clinical scores or physical examination [1, 15]. Instead, they can be proved by the long-term failure rate or proprioceptive function. However, despite the technological advances, there are still many limitations in accurately assessing proprioceptive function [15, 32].

Some of the previous ACL reconstruction procedures for which the term “remnant-preserving technique” was used were performed with type 4 ACL remnant tissue as categorized by Crain et al. [29] The method used in previous studies was not much different from single-bundle reconstruction, without repair or re-tensioning and merely leaving fibers on the tibial footprint [4, 8, 9]. Many of these studies reported no significant difference between remnant-preserving and conventional single-bundle reconstruction. However, for remnant tissue to play a role and properly function, (1) good quality of the remnant and (2) appropriate tension are necessary. Leaving only some remnant of the tibial side may have a synergistic effect on graft incorporation, synovialization, and revascularization; however, it is unlikely to significantly affect the stability and proprioception [15, 17, 19]. Rather, the risk of cyclops lesion could be increased [2, 19].

Longitudinal tension on a ligament results in compression of the connective tissue, leading to stimulation of the mechanoreceptors. Mechanoreceptors can also be stimulated by length changes, as well as by the rate of changes in tension and length [15, 17, 18, 33]. Therefore, it is necessary to create an environment in which mechanoreceptors can be adequately stimulated. Accordingly, “repair or re-tensioning with graft augmentation” may be a more appropriate method for effective maintenance and recovery of proprioception than simply “conserving” the remnant.

In this study, only the mechanical strength of the remnant fiber was assessed. In practice, however, as autogenic or allogenic graft tendons are used as the main ACL graft, the strength of remnant fibers is relatively insignificant. However, with the concept of additional fibers, it is reasonable to apply a method that can withstand as much force as possible [16, 19]. Further studies are needed to compare the mechanical strength of the single-bundle method alone and single bundle augmentation with remnant repair method.

This study has several limitations. First, the results were based on an in vitro biomechanical study and time-zero results and did not account for biological factors such as ligament incorporation, collateral ligament, posterior cruciate ligament, or the effect of the meniscus as a knee co-stabilizer. Also, we examined without cyclic loading. The compromised ACL is difficult to tolerate cyclic load by itself and is used as a concept of augmentation on the graft tendon. Thus, in previous studies about ACL remnant strength, only preload was applied and cyclic load was not applied [7, 22]. Therefore, in this study, only axial loading was performed to test with protecting of ACL remnants. Second, as previously noted, this study used porcine knees, which may not entirely correspond to human knees. Nonetheless, the key anatomic features and functional characteristics of the porcine model are similar to those of human knees [34]. Moreover, porcine models are commonly used in biomechanical studies of the meniscus [27, 35]. Third, we measured the mechanical strength with tensile loading at 30° flexion [7]; however, the actual human knee moves dynamically through a wide range of angles, rotation, and pivoting, and the axial tensile load used in this study might not reflect the behavior of the knee during real-life functional activities. Fourth, to maximally simulate real conditions, re-tensioning was applied after cutting the ACL at the femoral attachment. There is a possibility that the state of the surrounding soft tissues, such as synovium adhesion, may be different for each experimental subject. This may have affected the load-to-failure and stiffness measurements. Fifth, what we created was a complete femoral ACL “detachment” model, not a real “rupture” model. This issue should be taken into account because differences may exist between these two different injury patterns in terms of healing capacity [7, 36]. Sixth, we could not simulate actual condition of ACL reconstruction. During the ACL reconstruction procedure, the tibial side ACL remnant would be impaired. As introduced by Ahn et al. [1] and Noh et al. [37] with medial traction of the remnant using sutures and with final hand reaming technique could reduce additional injury of remnant ACL tibia footprint. Seventh, the suture material used in repair was No. 0 PDS. Each surgeon may prefer a different material (absorbable vs. non-absorbable) [1, 7, 12, 13] and the results may vary depending on the suture material used.

Conclusions
The results of this study suggest that loop stitch or triple stitch would be a better option for increasing the mechanical strength when applying remnant-preserving and re-tensioning reconstruction during ACL reconstruction.

Abbreviations
ACL: Anterior cruciate ligament; PDS: Polydioxanone suture

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Not applicable.

Authors’ contributions
DJR and KBK performed the investigation and interpretation of data and drafting of manuscript. DHH performed the investigation and interpretation of data. SJP had substantial contributions to conception and design. JSP was responsible for acquisition and analysis of data and performed the statistical analysis. JHW is an experienced knee surgeon who designed and controlled this study. The authors have read and approved the final manuscript.
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Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study used fresh frozen porcine knees as derived as a by-product of the food industry which is not subject to Institutional Review Board or the Animal experimentation Ethics Committee approval. We obtained the porcine knees by Cellummed (Seoul, Korea).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest in the authorship and publication of this article.

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