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Original article

Biomechanical comparison of different tendon suturing techniques for three-stranded all-inside anterior cruciate ligament grafts

Jae-Sung Yoo [a], Sung Jae Lee [b], Ji Eun Jang [b], Youngwoong Jang [c], Chaneol Kim [c], Yong In [d,*]

[a] *Department of Orthopedic Surgery, Dankook University College of Medicine, Cheonan, Republic of Korea*
[b] *Department of Biomedical Engineering, Inje University, Gimhae, Republic of Korea*
[c] *Central R&D Center, Corentec Co. Ltd, Seoul, Republic of Korea*
[d] *Department of Orthopaedic Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-Daero, Seocho-Gu, Seoul 06591, Republic of Korea*

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ABSTRACT

Objective: In all-inside anterior cruciate ligament (ACL) reconstruction, it is usually difficult to obtain sufficient autologous semitendinosus tendon length for quadruple stranded graft in Asians, females, and those with short stature. The purpose of this study was to compare biomechanical properties of three different types of suture preparations for tripled graft and determine which method could achieve sufficient strength for ACL through in vitro study. The hypothesis of this study was that suturing with a rip-stop (RS) stitch for tripled-strand graft would lead to stronger mechanical properties than suturing with buried-knot four sutures.
Methods: Twenty-four bovine digital extensor tendons harvested from forelimbs were prepared for tripled-strand graft in three different ways: (1) buried-knot four sutures, (2) two RS sutures, and (3) four RS sutures. These grafts were directly connected to cylindrical metal rods of a tensile testing machine. All specimens underwent cyclic loading followed by a load-to-failure test. Preparation time, elongation, stiffness, and ultimate failure load were compared.
Results: For biomechanical comparison, the group with buried-knot four sutures was excluded because six (75%) specimens failed during the cycle load test. The group with four RS sutures showed lower total elongation (two RS sutures: 8.42 ± 5.28 mm; four RS sutures: 3.86 ± 0.83 mm, $p = 0.030$), higher stiffness (two RS sutures: 247.28 ± 53.39 N/mm; four RS sutures: 329.27 ± 55.56 N/mm, $p < 0.001$), and higher ultimate failure load (two RS sutures: 567.74 ± 60.50 N; four RS sutures: 736.46 ± 32.50 N, $p = 0.009$). The most common failure mechanism of triple stranded graft was tendon split across sutures.
Conclusion: The method with four RS sutures showed sufficient strength for triple stranded graft for all-inside ACL reconstruction without increasing preparation time.
Level of evidence: III, controlled laboratory study.

1. Introduction

Successful anterior cruciate ligament (ACL) reconstruction depends on various factors, including surgical technique, graft selection, and type of fixation [1,2]. Hamstring tendon grafts have become particularly popular because of their low donor site morbidity and adequate biomechanical properties [3–5].

Recently, all-inside ACL reconstruction technique using cortical suspensory fixation devices with adjustable-graft loop length on both tibia and femur has been introduced with satisfactory clinical outcomes [6–8]. In this technique, the harvested single semitendinosus tendon is looped into four strands and linked to tibial and femoral ACL TightRope Reverse Tension devices (Arthrex, Naples, FL, USA) [6–9]. Using this device, the tension of the graft can be increased theoretically even after graft fixation using an adjustable length of the graft loop [10]. Therefore, the strength of the graft-adjustable loop construct is important for successful all-inside ACL reconstruction [6–9].

Graft length is important for all-inside ACL reconstruction in order to obtain complete filling of the graft in femoral and tibial sockets with proper intra-articular length [10]. Graft length of intratunnel should be at least 20–25 mm to ensure bone graft incorporation [10]. Qi et al. [11] have found that biomechanical strength and histologic maturity after ACL reconstruction are delayed if the

* Corresponding author.
E-mail address: iy1000@catholic.ac.kr (Y. In).

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graft length of intra-tunnel is less than 15 mm. Shaffer et al. [12] have measured intra-articular graft distance from tibial to femoral attachment and found that the average value is 26.3 ± 3.0 mm. Therefore, 66.3–76.3 mm is the target length of graft for proper intratunnel positioning. Lubowitz et al. [9] have described that if the semitendinosus tendon length is not greater than 270 mm, the final length of quadrupled strand is not more than 75 mm. Haber et al. [13] have reported that if the single strand tendon length is 271–280 mm, the predicted quadrupled length is only 65.5–66.8 mm. As a result, more than 270 mm of single strand tendon length is necessary to obtain proper length of quadruple strand graft. Fabbri et al. [10] have recommended triple-stranded graft for total semitendinosus length of less than 260 mm.

Several studies have suggested a minimum graft tendon diameter of 7 mm to guarantee good results in single-bundle reconstruction [14,15]. Therefore, a four-stranded graft with semitendinosus tendon is widely used in all-inside ACL reconstruction to achieve sufficient graft diameter [16–18]. However, graft length is correlated with total semitendinosus tendon length. It is not always sufficient to achieve a quadrupled graft for all-inside ACL reconstruction [10]. Chiang et al. [19] have reported that Chinese people have significantly shorter hamstring tendons than Caucasians (predicted length: 28.9 cm in Caucasians and 26.1 cm in Chinese) [19]. Moreover, the length of graft is affected by height, weight, and body mass index (BMI) [20]. Therefore, triple-stranded graft is sometimes inevitable, especially in East Asians, females, and those with short stature.

Suture strength sufficient to hold suspensory devices is more crucial in a triple-stranded graft than that in a quadruple stranded graft. This is because free ends of a triple stranded graft are located at both sides whereas free ends of a quadruple stranded graft are located at just one side. Therefore, more secure suture method is especially necessary for triple stranded graft preparation of all-inside ACL reconstruction. We developed a new suturing method: a rip-stop (RS) stitch for triple-stranded graft loop. There is a paucity of data on biomechanical evaluations of the strength of the linkage of the graft loop of triple-stranded semitendinosus graft for all-inside ACL reconstruction [10]. Therefore, the aim of this study was to compare biomechanical properties of three different suturing methods (buried-knot four sutures, two RS sutures, and four RS sutures) for tripled graft loop. The hypothesis of this study was that suturing with an RS stitch would lead to stronger mechanical properties than suturing with buried-knot four sutures.

2. Materials and methods

2.1. Specimen preparation

Testing was performed using 24 bovine digital extensor tendons divided into three groups (eight specimens per group). Extensor digitorum communis tendons were harvested from adult bovine forelimbs known to have structural and viscoelastic properties similar to those of human hamstring tendons [21]. Specimens were stored at −20 °C and thawed at room temperature for two hours prior to graft preparation and biomechanical testing [22]. They were kept moist until testing by being wrapped in tissue paper soaked with Ringer's solution and stored in sealed polyethylene bags [22]. These tendons were cut to a single-stranded length of 220 mm in order to reach a triple folded graft length of approximately 70 mm for the surgical all-inside graft-link technique [6–9]. Because we used bovine tendons, all grafts were adjusted to have a diameter of 8 mm with a graft-sizing block. Grafts with diameter less than 8 mm were not used for this study while grafts with diameter of more than 8 mm were trimmed to fit 8 mm diameter for biomechanical evaluation under the same condition.

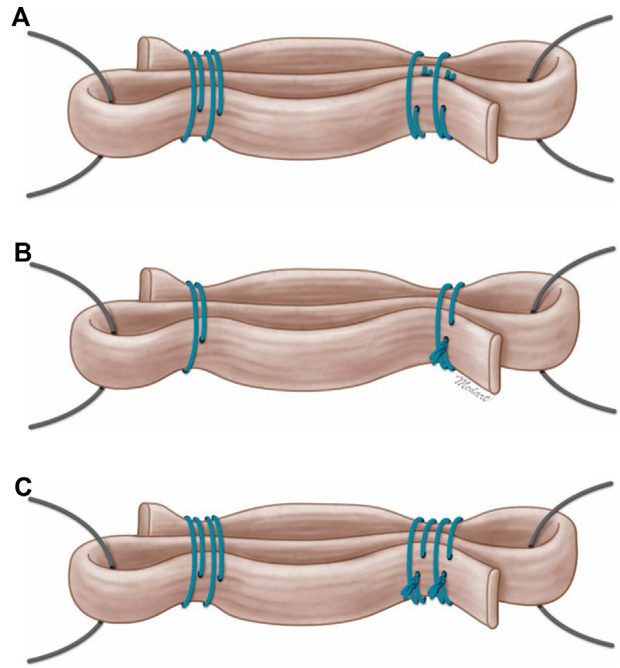

Fig. 1. The tendon was looped as triple-stranded with free ends on different sides. A. Conventional four buried-knot sutures; B. Two rip-stop sutures; C. Four rip-stop sutures.

All specimens were divided into three groups according to suture method: buried-knot four sutures, two RS sutures, or four RS suture (Fig. 1). All tendons were folded as three-stranded graft and secured with sutures (Ethibond N° 2, Ethicon Inc. Somerville, NJ, USA) placed 5 mm from both ends of graft in two RS sutures or placed 5 mm and 15 mm from both ends of graft in buried-knot four sutures or four RS sutures. In the group with buried-knot four sutures, a buried-knot technique was used. Each stitch passed through each strand of the graft while the suture limb was wrapped around the bundle once [6].

The RS suture method in graft preparation used in this study was modified from the Mason-Allen technique.[23,24] The suture started from the free end of tendon. It passed through all strands. The suture limb was also wrapped around the bundle once. An RS stitch was then performed at the terminal side of free end tendon from the start point (Fig. 2). The suture was tied with five manual knots. The time required to complete implantable graft, initial graft preparation time, and graft suturing time were measured and compared among the three techniques.

2.2. Biomechanical testing

Following preparation, the graft was placed at a servohydraulic material testing machine MTS 858 Bionix (MTS system Corp., Minneapolis, MN, USA). Ends of the graft were loaded in custom designed jigs rigidly fixed to the frame and the actuator of the material testing machine (Fig. 3). The graft was vertical to the loading axis of the machine so that the force was applied in line with the graft to test the worst load scenario. To stabilize mechanical properties of the graft, a static pre-conditioning of 50 N was applied for 5 min followed by a load to failure test. It was then loaded between 50 and 250 N for 1000 cycles at a frequency of 1 Hz [25–28]. In accordance with the cyclic loading protocol, a load-to-failure test at 50 mm/min was carried out.[25–28]. The maximum load was limited to 750 N because 1 kN load cell was used in this study. Cyclic loading and load-to-failure data were recorded using MultiPurpose TestWare software (MTS system Corp., Minneapolis, MN, USA) at a

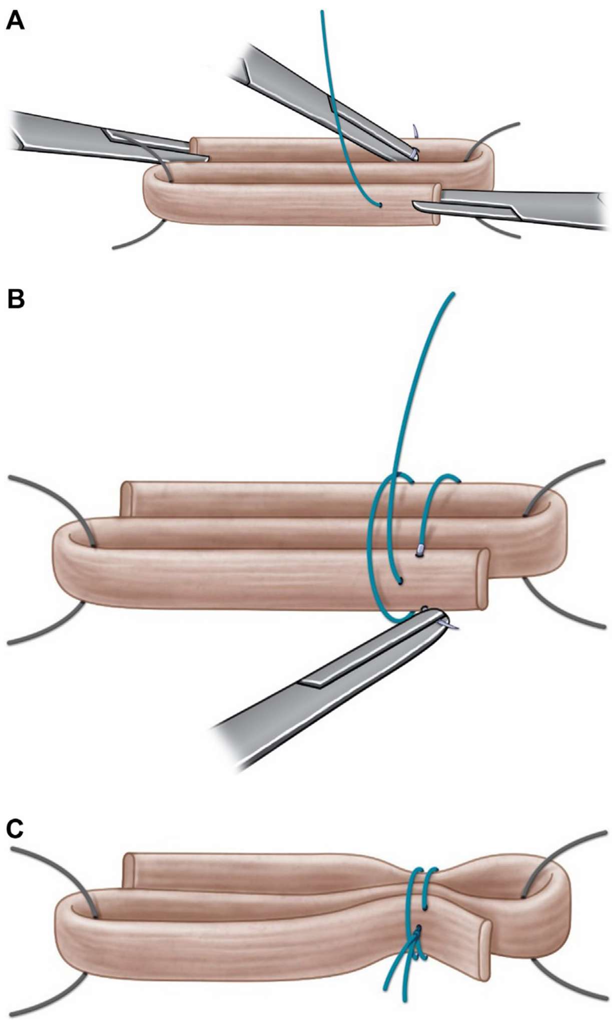

Fig. 2. A. Rip-stop (RS) suture begins by passing through all strands from the free end of tendon; B. The suture limb is wrapped once around bundles. RS stitch is performed at the terminal side of free end tendon from the start point; C. After RS stitch, the suture is tied with 5 manual knots.

sampling rate of 500 Hz. The resolution of the used measurement system was 1 N for the force and 0.075 mm for the displacement. In the loading protocol, graft elongations (mm) during preconditioning, the first five cycles (initial elongation), and cycles 1–1000 (total elongation) were evaluated. In the final load-to-failure test, ultimate failure load (UFL) (N) and pull-out stiffness (N/mm) were determined. The mechanism of failure was noted as well.

2.3. Statistical analysis

All statistical analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, NY, USA). The Kolmogorov–Smirnov test was used to check whether data had normal distribution. Graft suturing time, preconditioning elongation, and initial elongation were compared using analysis of variance (ANOVA) with Bonferroni post-hoc test in order to compare three groups because those factors showed normal distribution. However, total elongation, stiffness, or UFL could not be compared among the three groups because 6 specimens in the group with buried knot 4 sutures could not finish the test. As a result, total elongation, stiffness, and UFL were analyzed using independent t-test to compare two RS sutures and four RS sutures groups. Significance level was set at $p < 0.05$.

Fig. 3. Experimental set-up showing that the graft is directly connected to the material testing machine using cylindrical metal rods.

3. Results

The mean duration from the start of graft preparation to the beginning of graft suturing was identical for all three techniques (5.81 ± 1.80 min). The time for suturing was significantly shorter for the two RS sutures (3.63 ± 0.58 min) than that for the buried-knot four sutures (6.40 ± 0.80 min, $p < 0.001$) or four RS sutures (7.41 ± 0.99 min, $p < 0.001$), but not significant between groups with buried-knot four sutures and four RS sutures ($p = 0.115$, Table 1).

There were no significant differences in preconditioning elongations or initial elongations among the three groups (Table 1). In the group with buried-knot four sutures, only two (25%, 2/8) specimens could finish 1000 cycles while the others (75%, 6/8) failed due to tendon slippage across the suture during cycles (mean: 169.17 cycles; range: 0–385 cycles). Due to failures of tendons in the group with buried-knot four sutures, total elongation, stiffness, or UFL could not be compared among the three groups. The four RS sutures showed superior results in total elongation, stiffness, and ultimate failure load compared to the two RS sutures ($p = 0.030$, $p < 0.001$, and $p = 0.009$, respectively, Table 1).

In the buried-knot four sutures, all specimens failed due to tendon slippage across the suture (Fig. 4, 4A). In the two RS sutures, 75% (6/8) failed due to tendon slippage across the suture and 25% (2/8) failed due to loosening of sutures (Fig. 4B). In the four RS sutures, 25% (2/8) failed due to tendon slippage across the suture while 75% (6/8) of specimens endured the maximum load (750 N) used in this study (Fig. 4C).

Table 1
Biomechanical properties of specimens and relative *p*-values.

Technique		Graft suturing time (min)	Preconditioning elongation (mm)	Initial elongation (mm)	Total elongation (mm)	Stiffness (N/mm)	Ultimate failure load (N)
Four sutures	Mean ± SD	6.40 ± 0.80	13.21 ± 1.78	0.41 ± 0.11	[a]5.58 ± 0.58	[a]257.26 ± 29.80	[a]501.52 ± 124.56
	(range)	(5.50–8.00)	(10.30–14.73)	(0.32–0.62)	(5.17–5.99)	(236.18–278.33)	(413.44–589.59)
Two rip-stop sutures	Mean ± SD	3.63 ± 0.58	13.17 ± 1.58	0.36 ± 0.17	8.42 ± 5.28	247.28 ± 53.39	567.74 ± 60.50
	(range)	(3.00–4.60)	(10.97–14.75)	(0.01–0.58)	(5.52–20.92)	(155.93–321.76)	(499.13–640.07)
Four rip-stop sutures	Mean ± SD	7.41 ± 0.99	11.12 ± 2.26	0.40 ± 0.12	3.86 ± 0.83	329.27 ± 55.56	736.46 ± 32.50
	(range)	(6.00–9.20)	(7.62–14.39)	(0.28–0.58)	(2.93–5.12)	(243.30–425.23)	(657.16–750.00)
p-value		< 0.001	0.062	0.733	0.030	< 0.001	0.009

p-values of graft suturing time, preconditioning elongation, and initial elongation are compared with ANOVA test; *p*-values of total elongation, stiffness, and ultimate failure load between the group with two rip-stop sutures and the group with four rip-stop sutures are compared with an independent t-test except for comparison with the group with conventional four sutures; SD: Standard deviation.

[a] Mean values of two specimens among the eight that could finish 1000-cycle load test.

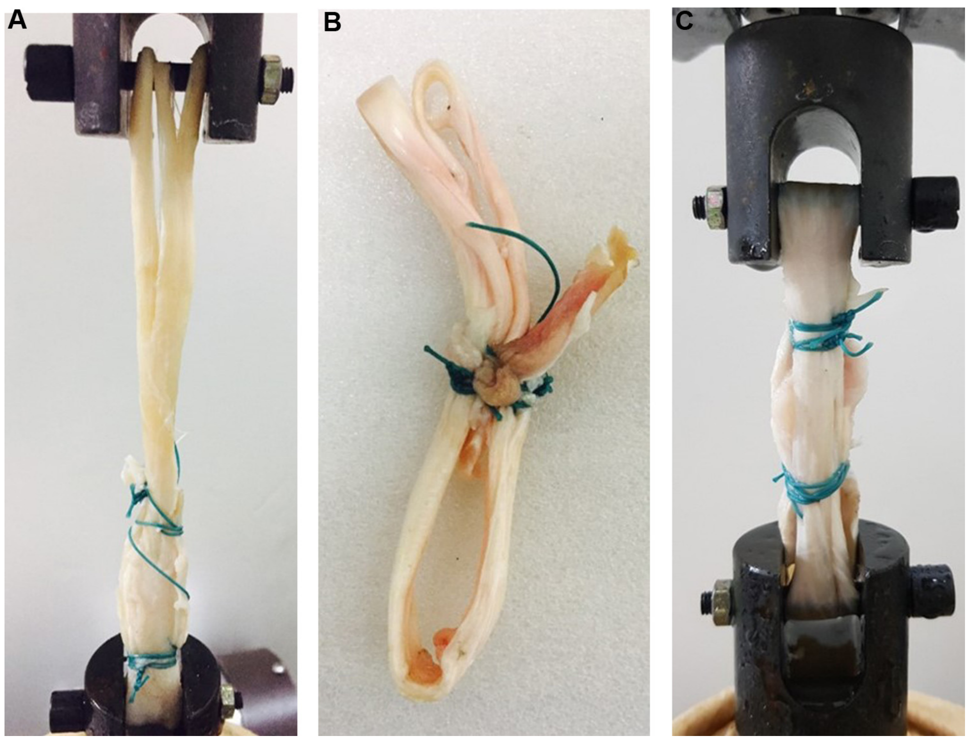

Fig. 4. Photograph showing failure mechanisms of graft. A. Loosening of sutures; B. Tendon slippage across the suture; C. No failure until maximum load (750 N).

4. Discussion

To the best of our knowledge, this is the first study to report biomechanical results of triple-stranded hamstring autografts according to preparation methods with new, simple, and secure RS sutures for all-inside ACL reconstruction. The most important finding of this study was that RS sutures for triple-strand graft showed satisfactory graft elongation and UFL without any increase in preparation time, supporting our hypothesis. Therefore, the RS suture method without bilateral whipstitch in triple-stranded graft is a very effective and useful method.

Graft length is important for all-inside ACL reconstruction in order to achieve complete filling on both femoral and tibial tunnels [10]. However, studies evaluating biomechanical properties of suturing methods in triple-stranded graft have been limited thus far.

Understanding the failure mechanism of graft is important for overcoming weak point of the graft. In the present study, 16 out of 18 (89%) (excluding six specimens that endured the maximum load of 750 N) failed due to tendon slippage across the suture. Therefore, free tendon end was suggested as being the weakest point of graft for suspensory fixation.

Lubowitz et al. [9] have reported a buried knot technique with a self-reinforcing suture. Mayer et al. [22] have also performed three stitches at free ends of a quadruple-stranded graft. However, free ends of a triple-stranded graft are located at different sides while free ends of a quadruple-stranded graft are located at the same side. As a result, whipstitches of a triple-stranded graft must be performed twice at each side, making the method ineffective and time-consuming. A variety of stitch methods have been proposed. Of them, RS suture has high ultimate tensile strength because it can improve resistance to suture cut-out [29–31]. RS suture is also an effective method for avoiding cinching. It can improve load to failure compared to simple or mattress stitch [32–34]. The RS suture used in the present study is a simple and effective method. Depending on activities involved, the load to graft tendon force in vivo ranges from 67 N to 454 N [10]. All specimens in the group with two RS sutures and the group with four RS sutures showed UFL higher than 454 N.

Elongation of the graft tendon should be kept to a minimum to avoid postoperative laxity [35,36]. Mayer et al. [22] have reported that the mean value of preconditioning elongation of buried-knot four sutures is 1.6 mm, with a mean initial elongation (after 5 cycles) of 2.7 mm, a mean total elongation (after 1000 cycles) of

Table 2
Comparison of biomechanical properties with previous studies.

Technique	Strands of graft	Total elongation (mm)	Ultimate failure load (N)
Two rip-stop sutures of this study	Triple	8.42 ± 5.28	567.74 ± 60.50
Four rip-stop sutures of this study	Triple	3.86 ± 0.83	736.46 ± 32.50
Four sutures of Fabbri et al. [10]	Triple	–	650.70 ± 27.41
Four sutures of Fabbri et al. [10]	Quadruple	–	767.02 ± 53.19
Two sutures of Mayer et al. [22]	Quadruple	7.0 ± 0.7	699.0 ± 87.0
Four sutures of Mayer et al. [22]	Quadruple	6.1 ± 0.6	766.0 ± 70.0

6.1 mm, and a mean UFL of 766 N through biomechanical study of quadrupled strand tendons. Mean values of the present study show higher preconditioning elongation (12.50 mm) and lower initial elongation (0.39 mm) than those of the previous research. Such differences might have stemmed from differences in study design. In the study of Mayer et al. [22], cortical button loops were adjusted to all specimens and failure mechanism was rupture of the cortical button loops for 96% (23/24) of specimens. The purpose of the present study was to evaluate biomechanical strength of tendon according to suturing method, not to evaluate the strength of cortical button loops. Therefore, all specimens of this study were directly connected to the material testing machine using cylindrical metal rods (Fig. 3). Thus, the initial length would be shorter than that in the previous study.

In the present study, 6 (75%) specimens in the group with buried-knot four sutures could not finish the cycle load test. The group with two RS sutures showed greater total elongation than quadruple-stranded tendons in the previous study [22]. Moreover, one specimen in the group with two RS sutures was elongated as 20.92 mm, although it finished fatigue test with UFL (500.89 N) higher than 454 N. Total elongation of the group with four RS sutures was less than that of quadruple-stranded tendons in the previous study [22]. However, the group with four RS sutures showed sufficient UFL comparable with the UFL of quadruple tendons in previous studies of Fabbri et al. [10] and Mayer et al. [22], although UFL was estimated without 1000 cycle load test in the study of Fabbri et al. [10] (Table 2). Therefore, the authors of this study believe that four RS sutures could be used as another option for graft preparation.

Fabbri et al. [10] have described that stiffness seems to be more affected by the viscoelastic property of the tendon itself rather than by the preparation method because there are no differences among three different configurations (half-quadrupled, tripled, and quadrupled). However, stiffness showed a significant difference between the group with two RS sutures (247.28 ± 53.39 N/mm) and the group with four RS sutures (329.27 ± 55.56 N/mm) in the present study ($p < 0.001$). The reason for such different results might be because the RS stitch method not only involved perpendicular wrapping as buried-knot, but also passed along the long axis of the tendon.

This study has several limitations. First, the maximum load was limited to 750 N because 1 kN load cell was used in this study. As a result, UFL of six specimens in the group with four RS sutures that did not rupture at 750 N was estimated to be 750 N without a precise ultimate failure load. Therefore, the mean value of UFL of the group with four RS sutures was underestimated in this study. Second, the in vitro axial load test is not similar to an in vivo study because femoral and tibial tunnels are not on pure axial alignment. Third, bovine tendons were used rather than human hamstring tendons. However, they are commonly used because their stiffness and viscoelastic property are not significantly different from those of human hamstring graft [21]. These results can be probably extrapolated to human ACL reconstruction. Finally, this study only investigated time zero in vitro biomechanical properties. Further in vivo study on correlation with clinical outcomes is necessary for accurate evaluation.

5. Conclusion

Triple-stranded graft is sometimes inevitable because graft length is not always sufficient to achieve a quadrupled graft. Suture strength in a triple-stranded graft is more crucial than that in a quadruple stranded graft because free ends of a triple stranded graft are located at both sides. RS sutures can be effective option with sufficient strength, especially for triple-stranded graft preparation. Four RS sutures at triple-stranded graft show sufficient strength, comparable with quadruple-stranded graft of previous studies without increasing preparation time.

Disclosure of interest

The authors declare that they have no competing interest.

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Contribution of the authors

Yong In, Youngwoong Jang, and Chaneol Kim designed this study; Sung Jae Lee and Ji Eun Jang performed biomechanical trials; J.S.Y. wrote the article.

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