Biomechanical Comparison of Graft Preparation Techniques for All-Inside Anterior Cruciate Ligament Reconstruction

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Background: All-inside anterior cruciate ligament reconstruction (ACLR) is an emerging technique used to treat ACL injuries. The all-inside technique uses a 4-stranded graft made from a single tendon that is looped on itself. The 4 strands of the graft must be secured to each other to become a closed-loop structure. Various suture configurations exist to secure the graft to adjustable loop devices, and there is a lack of data to support one technique over another. In addition to the primary sutures used to fasten the graft together, accessory sutures can be tied over the button as secondary fixation.

Purpose: To evaluate biomechanical properties of 4-stranded grafts prepared in 5 different configurations.

Study Design: Controlled laboratory study.

Methods: Porcine flexor tendons (N = 25) were prepared in 5 different configurations (n = 5 tendons per group): simple-interrupted sutures (unsecured fixation), side-to-side fixation with and without secondary fixation, and end-to-end fixation with and without secondary fixation. The grafts were put through the same mechanical testing protocol (cyclic loading, pull to failure) to measure average load at graft failure, average displacement at failure, average stiffness, and average elongation rate. Differences between graft preparation techniques were investigated using 1-way analyses of variance (ANOVAs) with post hoc *t* tests ($P < .05$).

Results: Significant 1-way ANOVAs for each biomechanical property were found. Unsecured fixation was the weakest graft preparation with the lowest stiffness (167 ± 12 N/mm), lowest ultimate failure load (637 ± 99 N), and highest elongation rate (0.0033 ± 0.0007 mm/s). End-to-end fixation without secondary fixation showed the highest ultimate failure load (846 ± 26 N), highest stiffness (212 ± 10 N/mm), and lowest rate of elongation (0.0025 ± 0.0001 mm/s). End-to-end fixation, both with and without secondary fixation, as well as side-to-side fixation with secondary fixation showed significantly higher ultimate failure loads than grafts with unsecured fixation. End-to-end fixation performed better than side-to-side fixation; however, for most variables, the difference was not statistically significant. Secondary fixation did not provide significant improvement.

Conclusion: The all-inside ACL graft with simple-interrupted sutures is biomechanically inferior to a graft that has its free ends secured to the adjustable tibial loop. Adding secondary fixation to the tibial button does not significantly change the biomechanical properties. Further clinical studies are required to determine whether these findings translate into differences in clinical outcome.

Clinical Relevance: All-inside ACLR is gaining popularity in hamstring ACL reconstructive techniques. These results provide surgeons with guidance on the best graft preparation method when using a single quadrupled hamstring tendon graft.

Keywords: all-inside ACL reconstruction; ACL graft sutures; biomechanical testing; adjustable loop fixation

The all-inside anterior cruciate ligament reconstruction (ACLR) technique uses adjustable-length, looped cortical button devices for fixation of the graft on both tibial and femoral ends. Typically, a quadrupled semitendinosus hamstring tendon graft is used because of its favorable biomechanical properties over other graft materials[4,5,9,10,13] and similar elasticity and force elasticity curve to the native ACL.[15] Outcome studies[5,14,19] suggest that subjective and objective outcomes are comparable with a standard quadrupled semitendinosus and gracilis hamstring ACLR using suspensory or aperture fixation. The potential benefits of this newer technique include gracilis tendon preservation, larger diameter grafts, smaller skin incisions, and less postoperative pain.[2,6,12,13]

The quadrupled semitendinosus graft must be secured to itself and reinforced with sutures to become a closed-loop structure. Different suture configurations exist to secure the tendon to itself. The currently recommended technique is a graft with 2 interrupted sutures on both the tibial and femoral ends.[3,11,14,16] Alternatively, some surgeons secure

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the 2 free ends of the graft to the adjustable loop using side-to-side fixation or end-to-end fixation of the 2 free ends. The side-to-side or end-to-end sutures can be kept long and passed through the tibial cortical button and then tied as secondary fixation, but this adds to the suture load and preparation time. The added strength of a graft with these secondary fixation sutures is not clear.

The purpose of the study was to evaluate the biomechanical properties of the all-inside graft preparation technique using 5 different suture configurations: simple-interrupted sutures, side-to-side fixation with and without secondary fixation, and end-to-end fixation with and without secondary fixation.

We hypothesized that a graft with side-to-side or end-to-end fixation would have superior biomechanical properties compared with a graft with only interrupted sutures. In addition, we hypothesized that tying accessory sutures to the tibial button as secondary fixation would yield a graft with superior biomechanical properties in comparison with a graft without.

METHODS

A total of 25 porcine digital flexor tendons divided into 5 groups were used for mechanical testing. Porcine tendons were chosen because of their similar structure and viscoelastic properties to human semitendinosus tendons.[7,8] The porcine specimens were not frozen before graft preparation. The 4 stranded grafts were trimmed to fit through a 12 mm–diameter cylinder. The grafts prepared in this study are larger than the diameter of a typical ACL graft. During graft preparation, there was concern that trimming the tendons down to a typical diameter risked compromising the integrity of the tendon fibers. As a result, we decided to leave the diameters larger because we controlled for the diameter, and the comparison between groups was the most important for our study. The tendons were cut to a length of 200 mm to achieve a 4-stranded graft length of approximately 50 mm.[4,10] The prepared grafts were stored at −20°C and thawed at room temperature for 1 h before biomechanical testing.

Graft Preparation

Suture loops and buttons (Arthrex Tightrope RT; Arthrex Inc.) were used on both the tibial and the femoral ends to suspend the grafts. The individual graft types (Figure 1) were prepared as follows:

Graft A (unsecured fixation): The tendon was folded over the tibial suture loop symmetrically. Both free ends were passed through the femoral suture and doubled in alternating directions, yielding a quadrupled graft. The 4 strands of the graft were tied together with 4 No. 2 polyethylene-polyester braided suture (FiberWire; Arthrex) simple-interrupted sutures. Starting from the inside of the graft, the stitch was passed outward through 3 of the 4 graft strands. The suture was then wrapped around the graft and passed through the fourth layer, ending at the start of the suture and creating a self-reinforcing noose.[9] Of note, this is currently the recommended technique for graft preparation in an all-inside ACLR.

Graft B (secured side-to-side fixation): The tendon was folded over the tibial suture loop symmetrically, and both free ends were doubled and passed through the femoral suture loop in the same direction, yielding a quadrupled graft. The free ends of the graft were whipstitched together with 3 throws up and 3 throws down in a side-to-side manner with No. 2 polyethylene-polyester braided suture over a length of 20 mm. The needle was removed, and 1 end of these side-to-side sutures was passed through the suture loop on the tibial side and tied to the other suture (Figure 2). The 4 strands of the graft were then secured with 4 No. 2 polyethylene-polyester braided interrupted sutures as in Graft A.[9]

Graft C (secured end-to-end fixation): The tendon was folded over the tibial suture loop symmetrically, and both free ends of the graft were passed through the femoral suture loop in alternating directions, yielding a quadrupled graft. One free end of the graft was passed again through the tibial loop, and the free ends of the graft were held end-to-end and whipstitched with a single No. 2 polyethylene-polyester braided suture over a length of 20 mm on each limb using 3 throws up and 3 throws down. One limb was passed through the suture loop on the tibial side and tied to the other limb, yielding a secured end-to-end graft (Figure 1). The 4 strands of the graft were then secured with 4 No. 2 polyethylene-polyester braided interrupted sutures as in Graft A.[11,16]

Graft D and Graft E were prepared the same way as Graft B and Graft C, respectively, except that the suture ends of the side-to-side and end-to-end sutures were passed through the tibial button and tied; however, the 2 suture tails were left long for tibial-sided secondary fixation.

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Ethical approval for this study was waived by the University of Alberta Research Ethics Office.

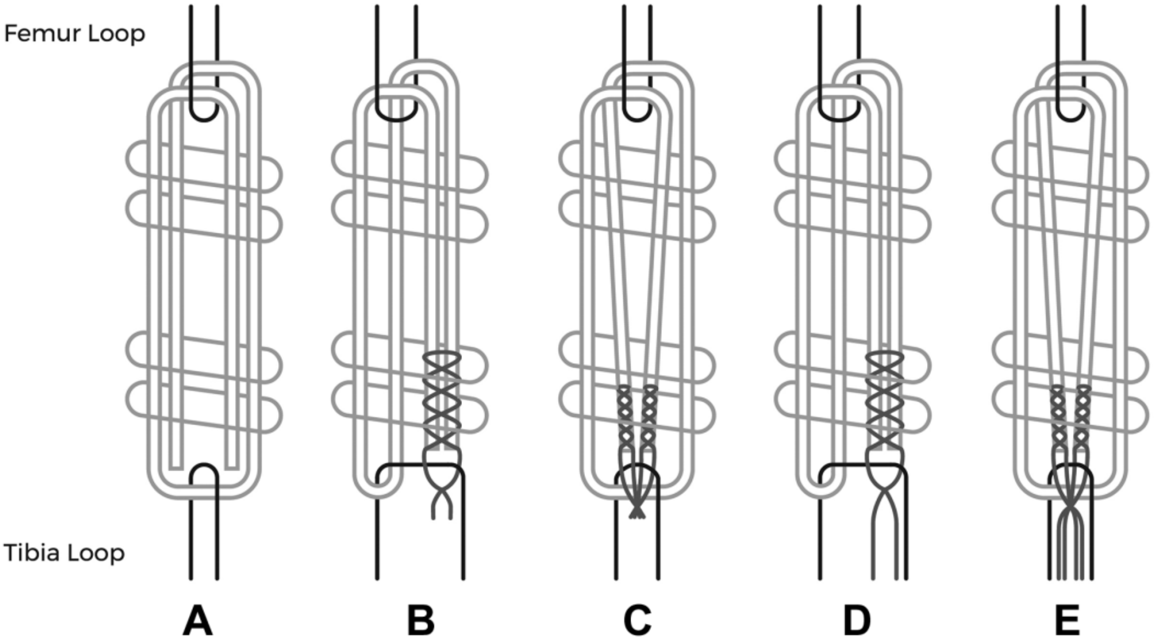

Figure 1. Illustration of graft preparation techniques. Graft A = unsecured fixation, Graft B = secured side-to-side fixation, Graft C = secured end-to-end fixation. Grafts D and E were prepared the same way as Grafts B and C, respectively, but the suture tails were left long for secondary fixation.

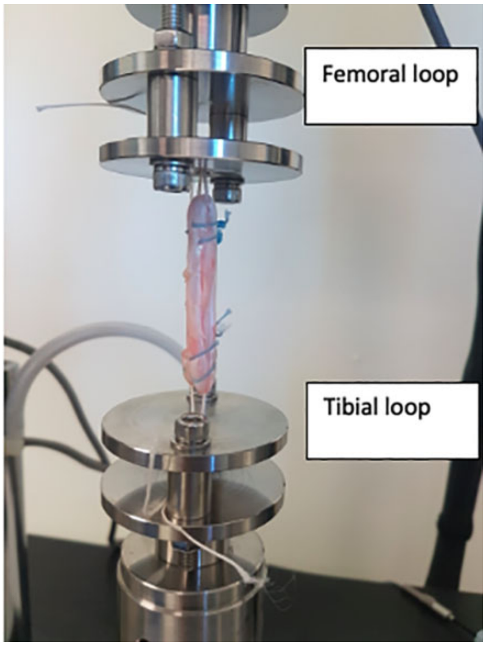

Figure 2. Mechanical test setup with an example graft fixed into the testing machine.

Mechanical Testing

Mechanical testing was performed on the 25 prepared grafts using a Bose ElectroForce 3510 mechanical testing machine (TA Instruments). Each graft was fixed on both ends with the Arthrex Tightrope RT and button device, with the graft aligned with the loading axis (Figure 2). The grafts were preloaded at a rate of 1 N/s up to 20 N, followed by a pretensioning phase in which the grafts were tensioned at 20 N for 5 min. The grafts then underwent cyclic preconditioning from 20 to 50 N at 0.1 Hz for 10 cycles, followed by cyclic loading from 50 to 250 N at 1 Hz for 500 cycles and last pull to failure at a rate of 20 mm/min. The mechanical testing protocol is similar to other studies.[13,18]

During the cyclic loading phase, the elongation rate (mm/s) was calculated as the slope of the linear portion of the displacement (elongation) versus time curve. The elongation during the cyclic loading phase (mm) was calculated as the difference in displacement between the first and last valleys of cyclic loading. During the load-to-failure phase, the stiffness (N/mm) was calculated as the initial slope of the linear portion of the load-displacement curve, and the ultimate failure load (N) was recorded. The elongation during the load-to-failure phase (mm) along with the total elongation (cyclic loading and load to failure combined, mm) was calculated. The method of failure was recorded as either rupture at the tendon to suture interface or rupture at the button loop interface. Both the load and displacement were measured via the mechanical testing machine. Thus, the elongation measurements include the combined elongation of both the graft and the button loops.

Statistical Analysis

Descriptive statistics (mean ± SD) were determined for each variable of interest. Differences in biomechanical properties between graft preparation techniques were

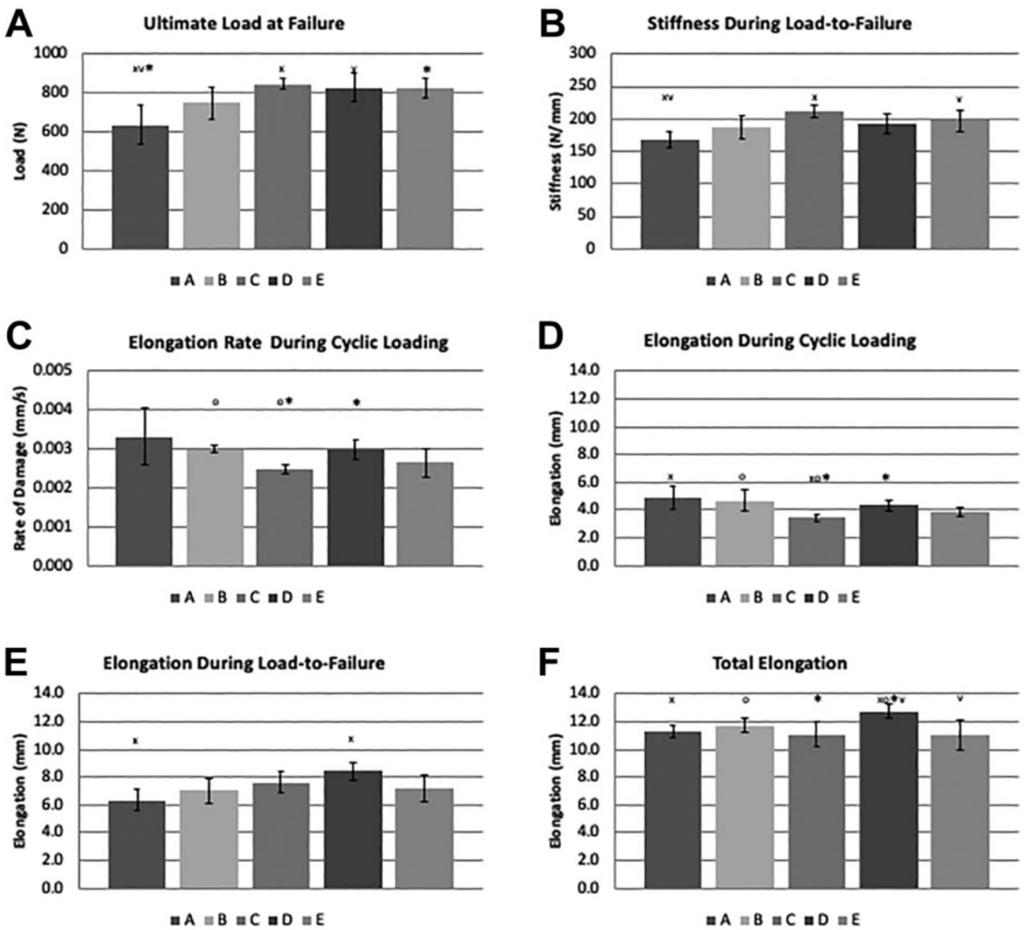

Figure 3. Average biomechanical properties for each graft preparation technique. Error bars indicate 1 SD. Corresponding symbols over the bars indicate statistically significant differences by post hoc *t* tests ($\alpha = .05$). *A* to *E* denote the different grafts constructs: Graft A = unsecured fixation, Graft B = secured side-to-side fixation, Graft C = secured end-to-end fixation. Grafts D and E were prepared the same way as Grafts B and C, respectively, but the suture tails were left long for secondary fixation.

assessed with a 1-way analysis of variance (ANOVA). Significant effects were evaluated with post hoc *t* tests using the Benjamini-Hochberg procedure for controlling the false discovery rate with multiple comparisons.[3] The required sample size (n = 5 per group) was estimated a priori based on the resource equation.[1] Post hoc power analysis was conducted using the 1-way ANOVA effect size for the ultimate load at failure.

RESULTS

The 1-way ANOVAs for each biomechanical property were found to be significant (ultimate failure load, $P < .001$; stiffness, $P = .002$; elongation rate during cyclic loading, $P = .021$; cyclic elongation, $P = .004$; load-to-failure elongation, $P = .011$; total elongation, $P = .007$). Post hoc analysis indicated a between-group ANOVA effect size of 0.60 for the ultimate load at failure. Based on a sample size of n = 5 per group, this resulted in a power estimate of 55%. To achieve a power of 80%, it was estimated that a sample size of n = 8 per group would be needed.

Ultimate Failure Load

Graft C (secured end-to-end fixation) had the highest average ultimate failure load (846 ± 26 N). Graft E (secured end-to-end fixation with secondary fixation) and Graft D (secured side-to-side fixation with secondary fixation) had nearly the same ultimate failure load (828 N and 829 N, respectively). Graft B (secured side-to-side fixation) had a lower ultimate failure load (749 N) and Graft A (unsecured fixation) had the lowest ultimate failure load (637 ± 99 N) (Figure 3A). Graft A had a significantly lower ultimate failure load than Graft C ($P = .002$), Graft D ($P = .009$), and Graft E ($P = .005$). Graft B had a lower ultimate failure load than Graft C; however, the differences were not statistically significant after Benjamini-Hochberg correction ($P = .036$). Further, there were no statistically significant differences in ultimate failure load between Grafts C, D, and E.

Stiffness

The stiffness of the load-to-failure curve showed a similar trend to the ultimate failure load, with the highest average stiffness exhibited by Graft C (212 ± 10 N/mm), followed by Graft E (198 N/mm), Graft D (192 N/mm), Graft B (187 N/mm), and Graft A (167 ± 12 N/mm) (Figure 3B). Graft C was significantly stiffer than Graft A ($P < .001$). Graft E was significantly stiffer than Graft A ($P = .009$). There was no significant difference in stiffness between the other graft comparisons.

Elongation Rate During Cyclic Loading

Investigating the increase in displacement over adjacent cycles during the cyclic loading phase gives an indication of the elongation rate in the graft. Again, the same trend was observed, with Graft C having the lowest elongation rate (0.0025 ± 0.0001 mm/s) followed by Graft E (0.0027 mm/s), Graft D and Graft B (0.0030 mm/s), and finally Graft A (0.0033 ± 0.0007 mm/s) (Figure 3C). In this case, Graft C had a significantly lower elongation rate compared with Graft B ($P < .001$) and Graft D ($P = .002$). Graft C had a lower elongation rate compared with Graft A; however, the differences were not statistically significant after Benjamini-Hochberg correction ($P = .033$). Additionally, Graft E elongated less than Graft A, Graft B, and Graft D; however, none of these differences were statistically significant after Benjamini-Hochberg correction (E vs A: $P = .030$; E vs B: $P = .047$; E vs D: $P = .040$).

Elongation During Cyclic Loading

The total elongation during the cyclic loading phase provides an indication of the total damage accumulated in the graft. The elongation accounts for elongation of the tendon graft and the button loops connected to the mechanical testing machine. The average elongation during cyclic loading again followed the same trend, with Graft C having the lowest (3.44 mm), followed by Graft E (3.81 mm), Graft D (4.34 mm), Graft B (4.70 mm), and finally Graft A (4.89 mm) (Figure 3D. Graft C elongated significantly less than Graft A ($P = .007$), Graft B ($P = .009$), and Graft D ($P = .002$). Additionally, Graft E elongated less than Graft A ($P = .030$), Graft B ($P = .047$), and Graft D ($P = .040$); however, the differences were not significant after Benjamini-Hochberg correction. There was no significant difference in elongation between Graft C and Graft E ($P = .076$).

Elongation During Load to Failure

The elongation during the load-to-failure test provides an indication of the stretch in the graft before rupture. Again, this elongation includes displacement both within the tendon graft itself and within the button loops connected to the mechanical testing machine. Graft D showed the highest elongation during this phase (8.41 mm) followed by Graft C (7.60 mm), Graft E (7.19 mm), Graft B (7.02 mm), and finally Graft A (6.36 mm) (Figure 3E). Graft A elongated significantly less than D ($P = .001$) with no other significant differences between the graft groups.

Figure 4. Suture-tendon rupture from load-to-failure test.

Figure 5. Button loop rupture from load-to-failure test.

Total Elongation

The total elongation represents the elongation of the graft throughout the entire test (after preconditioning), including both the elongation during cyclic loading and the elongation during load to failure. Graft D had the highest total elongation (12.76 mm) followed by Graft B (11.72 mm), Graft A (11.28 mm), Graft C (11.05 mm), and finally Graft E (11.01 mm) (Figure 3F). In this case, Graft D had a significantly higher total elongation than Graft A ($P = .001$), Graft B ($P = .012$), Graft C ($P = .005$), and Graft E ($P = .010$). There were no significant differences in total elongation between Grafts A, B, C, and E.

Failure Mode

The failure mechanism for each graft sample was recorded as either rupture at the tendon suture interface or button loop interface (Figures 4 and 5). The method of failure for Graft A was tendon-suture rupture (n = 5). Graft B had 2 button loop ruptures and 3 tendon-suture ruptures. Graft C failure resulted from button loop ruptures (n = 5). Graft D showed 3 button loop ruptures and 2 graft ruptures. Graft E mainly failed at the button loop (n = 4).

DISCUSSION

This study presents a novel biomechanical analysis of 5 different graft preparation techniques for the all-inside ACLR. The most important finding of this study is that an all-inside ACL graft with only interrupted sutures on both the tibial and the femoral ends is biomechanically inferior to a graft that has its free ends secured to the adjustable tibial loop. The second most important finding of this study is that when a graft is prepared with secured fixation to the tibial adjustable loop, keeping the suture tails long and tying them over the tibial button does not seem to significantly change the biomechanical properties of ultimate failure strength, stiffness, or elongation rate.

While the all-inside ACLR has many theoretical advantages, some surgeons remain skeptical about the biomechanical performance of a graft that is fixed on both ends under tension to cortical buttons. In our study, the average stiffness during load to failure of the fixed grafts (Graft B-Graft E) ranged from 187 to 212 N/mm, which is within the range of values reported for native ACL,[18] typical ACLR graft constructs,[7,13] and both porcine and bovine tendon grafts.[7,18] The average ultimate failure load of the fixed grafts ranged from 749 to 846 N. This is similar to the range of values reported for a quadrupled semitendinosus graft reported by Pailhé et al[13] (630.82 ± 239.15 N; range, 408.13-1123.44 N). This is on the lower end of typical values reported elsewhere for native ACL (739-2300 N)[17] as well as bovine and porcine tendon grafts.[7,13,18] For example, Vertullo et al[18] reported ultimate failure load values between 767 and 1097 N for quadrupled bovine tendon graft constructs.

Unsecured fixation utilizing only interrupted sutures to secure the 4 tendon limbs together is the current industry-recommended graft preparation technique. Interestingly, unsecured fixation yields the poorest quadrupled graft of the 5 tested in this study. Graft A (unsecured fixation) was found to have the lowest ultimate failure strength and stiffness along with the highest elongation rate and elongation during load to failure. Based on the findings of this study, we would recommend that surgeons using this technique consider other graft preparation techniques when using a quadrupled hamstring tendon graft for ACLR.

To avoid relying solely upon a single loop of tendon on the tibial side, surgeons should consider fixating the 2 free limbs of the tendon graft to tibial adjustable loop. Secured fixation utilizing end-to-end fixation to the adjustable loop yields the best biomechanical all-inside, quadrupled graft. Graft C (secured end-to-end fixation) was found to have the most favorable biomechanical properties compared with the other graft preparation techniques. However, some of the superior differences found were not statistically significant because of the small sample size and large number of comparisons.

End-to-end fixation seems to be stronger than side-to-side fixation, although the differences did not reach statistical significance in this study. Without secondary fixation, the end-to-end fixation technique seemed to perform better than the side-to-side fixation technique in terms of ultimate failure load, stiffness, and elongation rate during cyclic loading. However, statistical significance was not observed in our study, which is likely because of the small sample size and the large number of comparisons. With further samples, this trend may become statistically significant. However, this trend was not seen with the addition of secondary fixation. The only differences between the side-to-side and end-to-end techniques with the addition of secondary fixation were seen in the elongation during cyclic loading and total elongation.

Our hypothesis that incorporating accessory sutures as secondary fixation tied to the button would improve biomechanical properties was largely disproven. The addition of secondary fixation did not have a significant effect on any of the biomechanical properties tested, for end-to-end fixation. This was contrary to our hypothesis and suggests that the additional suture load and preparation time do not appear to increase the effectiveness of the graft when using end-to-end fixation. The addition of secondary fixation did not have a significant effect on the stiffness, elongation rate, or elongation during cyclic loading for side-to-side fixation, and a significant increase in pull-to-failure elongation and total elongation was observed. There was a slight increase in ultimate failure load; however, the difference was not statistically significant. The addition of secondary fixation to the button when using side-to-side fixation was not found to improve the biomechanical performance of the graft. Therefore, using secondary fixation should be questioned in the absence of strong biomechanical evidence and the increase in preparation time and suture load. However, clinical studies are required to determine if these biomechanical findings are consistent with clinical outcome.

Some surgeons have suspected that the “weak point” of the all-inside graft is found at the interface between the 4 tendon strands of the single tendon. This suspicion has been confirmed in recently published biomechanical study.[18] However, based on the results from our study, we propose that the “weak point” is predicted based on the graft preparation technique. Unsecured fixation utilizing only interrupted sutures to hold the 4 tendon limbs together will lead to failure at the tendon-suture interface, which is confirmed by the results in our study. This likely reflects failure of the single point of fixation on the tibial side: the single tendon loop to which the other limbs were fixed. The grafts prepared with end-to-end fixation largely failed by a rupture of the button loop (9/10). This suggests that the tendon graft itself, along with the sutures passed through the adjustable loop, was stronger than the adjustable button loop. Grafts prepared with side-to-side fixation failed with an equal mix of button loop rupture (5/10) and tendon-suture rupture (5/10). This likely suggests that the tendon-suture construct was closer in strength to the adjustable button loop. Whether failure at the tendon or adjustable loop is more or less desirable is not clear and the clinical significance unknown.

The strengths of this study include its being the first study to test the biomechanical properties of quadrupled tendon grafts for a group of grafts prepared in 5 different graft configurations. We were also able to test the effectiveness of the additional suture fixation into the tibial button implant, which has not been tested previously.

The limitations of this study include its laboratory design and the small sample size. The sample size of $n = 5$ per group was estimated a priori using the resource equation.[1] However, a post hoc power analysis revealed that the study may be underpowered, considering the large number of comparisons made (10 comparisons) for each biomechanical parameter. Based on the post hoc analysis of the ultimate load at failure, it is estimated that at least $n = 8$ samples per group may be required to achieve 80% power. However, even with a relatively small sample size, this study has shown significant differences between graft preparation techniques, warranting consideration in future studies and in clinical practice. Furthermore, while this study provides interesting biomechanical data, it does not necessarily reflect how each graft would perform in vivo. Whether the differences in biomechanical properties of the grafts will translate into differences in failure rates or clinical outcomes remains unclear. Additionally, porcine tendons were used, and although they have similar structure and viscoelastic properties with human tendons, it is difficult to know whether these results are generalizable to human tendon properties. Last, tendons were harvested from different porcine specimens, which is a limitation because different pigs may have different tendon properties that cannot be accounted for.

CONCLUSION

This study demonstrates that an all-inside ACL graft with only interrupted sutures on both the tibial and the femoral ends is biomechanically inferior to a graft that has its free ends secured to the adjustable tibial loop. When an all-inside ACL graft is prepared with secured fixation to the tibial adjustable loop, keeping the suture tails long and tying them over the tibial button does not significantly change the biomechanical properties of ultimate failure strength, stiffness, or elongation rate. Although further clinical studies are required to determine whether these biomechanical findings translate into differences in clinical outcome, these results may prompt surgeons who use all-inside ACL grafts to consider securing the free ends of the graft to the adjustable loop.

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