Original Article

Anterior Cruciate Ligament Reconstruction with 4-Strand Hamstring Tendon Construct May be Biomechanically Superior to 5-Strand Hamstring Tendon Construct When Using Femoral Suspensory Fixation

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Purpose: To compare stiffness, strain, and load to failure of 4- versus 5-strand hamstring anterior cruciate ligament reconstruction human tendon allografts with femoral suspensory and tibial interference screw fixation **Methods:** Allograft hamstring tendons were used to create 10 four-strand (4S) and 10 five-strand (5S) grafts. Grafts were fixed to a uniaxial electromechanical load system via a femoral cortical suspensory button and a bioabsorbable interference screw in bone analogue. Grafts were cycled from 100 Newtons (N) to 250 N for 1,000 repetitions at 0.5 hertz before load to failure testing. Cyclic displacement was defined as the difference in graft length from the first 20 to 30 cycles compared with the last 10 cycles. Trials were recorded on a high-definition camera to allow for digital image correlation analysis. **Results:** Cyclic displacement more than 1,000 cycles was significantly lower in the 4S compared with the 5S group (0.87 vs 1.11 mm, $P = .037$). Digital image correlation analysis confirmed that the fifth strand elongated more than the other 4 strands in the 5S constructs (6.1% vs 3.9%, $P = .032$). Load to failure was greater in the 4S compared with the 5S group but not statistically significant (762 vs 707 N, $P = .35$). Stiffness was similar between constructs (138.5 vs 138.3 N/mm, $P = .96$) **Conclusions:** Compared with cyclically loaded 4S hamstring grafts, the 5S grafts had significantly increased displacement over time in a model of femoral suspensory and tibial interference screw fixation. **Clinical Relevance:** Anterior cruciate ligament reconstruction with hamstring tendon autograft is a commonly performed surgery with excellent outcomes. It has been shown that graft diameter influences these outcomes. As surgeons use larger grafts, it is important to investigate how these constructs may affect the outcomes of surgery.

Anterior cruciate ligament reconstruction (ACLR) with hamstring (semitendinosus and gracilis) tendon autograft is a commonly performed surgery with excellent outcomes.[1,2] However, there is concern that hamstring ACLRs with smaller diameter grafts may carry increased risk of revision or poor outcome scores.[3-6] Two techniques have been described to increase the size of the obtained semitendinosus and gracilis autograft: allograft augmentation or additional folds of the graft to create a 5- or 6-strand graft. As numerous data have demonstrated increased rerupture rates of allograft augmentation for hamstring ACLR compared with autograft alone,[7-9] more attention is turning toward 5- and 6-strand techniques.

In the 5- and 6- strand hamstring autograft techniques, the semitendinosus and/or gracilis tendons are tripled over to create the additional strand(s). This technique has been found to consistently increase graft diameter more than a millimeter.[10] To date, clinical and biomechanical studies have not demonstrated any clear advantage of this technique over the standard 4-strand

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The authors report the following potential conflicts of interest or sources of funding: M.S.H. reports nonfinancial support from RTI and Smith & Nephew during the conduct of this study. Full ICMJE author disclosure forms are available for this article online, as supplementary material.

Received March 14, 2021; accepted March 11, 2022.

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2666-061X/21405

https://doi.org/10.1016/j.asmr.2022.03.008

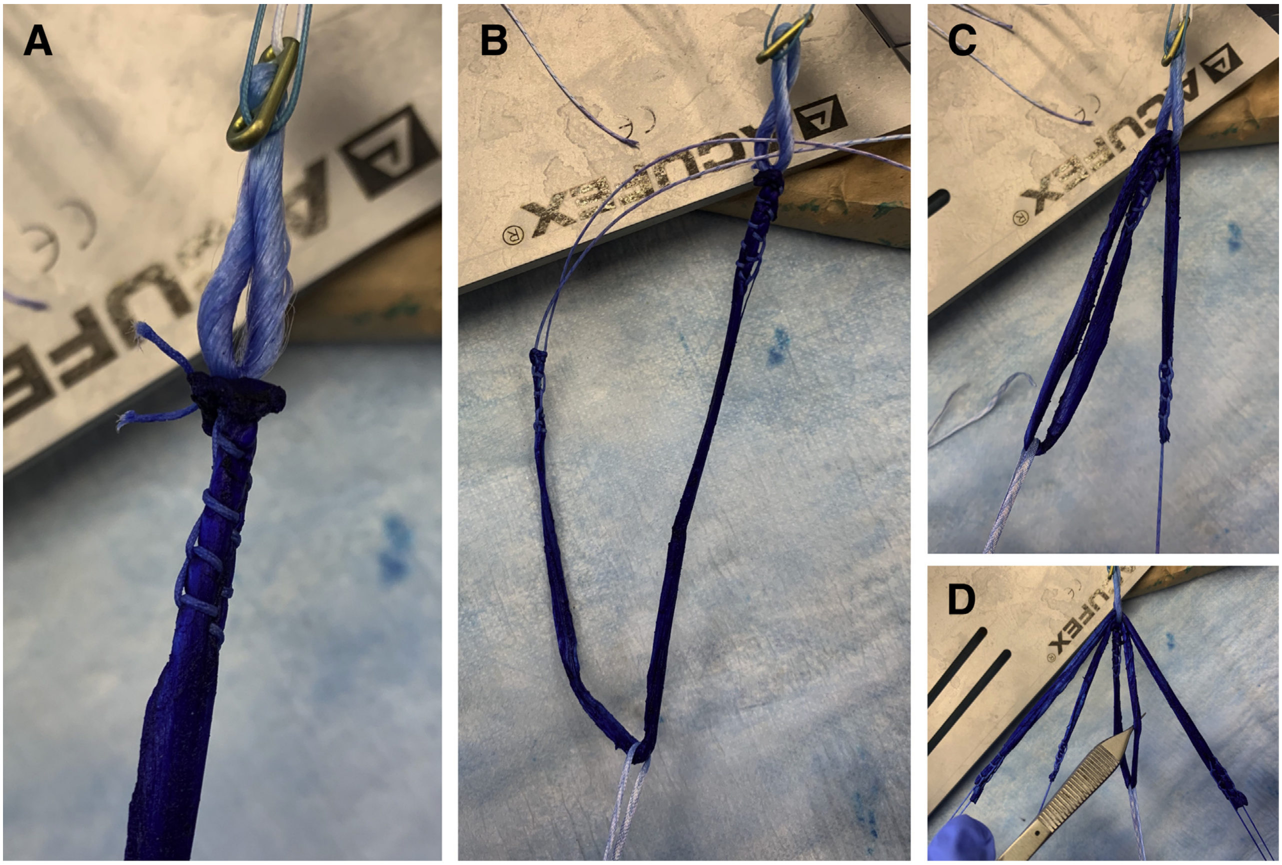

Fig 1. Creation of the 5-strand anterior cruciate ligament graft. One end of the whipstitched semitendinosus allograft is tied to the loop of the suspensory button with 6 square knots, then trimmed (A). The free end is then passed through the button (B). A suture tape is used distal the suspensory loop to divide the graft into 3 equal limbs (C). The gracilis allograft is then passed through the suspensory loop to create the 5-strand graft (D). In this example the construct was dyed with methylene blue, and after mounting it was speckled with white paint for digital image correlation analysis.

hamstring graft.[11,12] However, existing biomechanical studies are limited as they do not closely replicate the in vivo scenario, either due to graft choice (bovine vs human tissue), omittance of the additional doubled-over tendon (i.e., 3-strand instead of 5-strand), or omittance of standard tibial fixation.[13-16] The purpose of this study was to compare stiffness, strain, and load to failure of 4- versus 5-strand hamstring ACLR human tendon allografts with femoral suspensory and tibial interference screw fixation. Our hypothesis was that in this biomechanical model, the extra fifth strand would demonstrate better stiffness, strain, and load to failure than the 4-strand constructs.

Methods

This was a controlled laboratory study approved by the University of Washington institutional review board. Frozen human donor semitendinosus and gracilis allografts (RTI Surgical, Alachua, FL) were used. Allografts were sterilized and processed with proprietary BioCleanse radiation-sparing treatment and kept frozen until the time of experimentation. None of the grafts were expired at the time of testing. The grafts were thawed to room temperature and visually inspected for gross defects. Any tendons with gross injury were excluded. Throughout preparation and experimentation, the tendons were kept moist with normal saline. Graft length varied from 200 to 260 mm (average 233 mm), with mean donor age of 54.5 years.

Graft Preparation

Group 4S consisted of ten 4-strand constructs. One gracilis and one semitendinosus tendon were used for each construct. Each tendon was whipstitched 3 cm at either end with #2 high-strength polyethylene suture (ULTRABRAID; Smith & Nephew, Andover, MA), and then folded over a 20-mm fixed-loop cortical button (ENDOBUTTON; Smith & Nephew.) to create a 4-strand graft. Grafts were pretensioned on a graft preparation station at 60 N and then sized.

Group 5S consisted of ten 5-strand constructs. Only semitendinosus tendons greater than 240 mm in length were selected, to allow for enough length when tripled

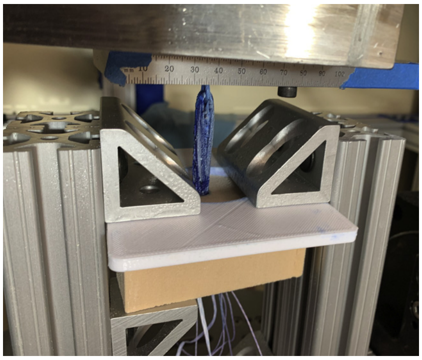

Fig 2. Robot setup for biomechanical testing of the 4- and 5-strand anterior cruciate ligament grafts. The suspensory button was suspended from a metal plate while the tibial block was clamped distally. The graft was secured to the tibial block with an interference screw. The ruler was used for scale during subsequent video analysis.

over. One gracilis tendon also was used for each construct. Tendons were whipstitched at each end as in Group 4S. The semitendinosus was tied to the cortical button loop with a surgical knot of 6 square knots and then looped over (Fig 1 A-C). A smooth flat suture tape (ULTRATAPE; Smith & Nephew) was then placed on the opposite folded end of the tendon. The gracilis tendon was folded in half over the button loop, thus combining to make a 5-strand graft (Fig 1D). Grafts were pretensioned on a graft preparation station and then sized.

Once sized, the grafts were mounted to the simulated tibial fixation. A rigid reamer of corresponding size was used to drill into polyurethane foam bone analogue (Pacific Research Laboratories, Vashon, WA) of 30 mm depth and 320 kg/m^3 (20 pounds per cubic foot) density. This density matches cancellous bone and similar polyurethane foam has been used and validated in previous studies on interference screw fixation.[17] The cortical button and graft were passed through the bone analogue, suspended from a fixed metal plate and then each graft strand was held on tension distally while inserting a bioabsorbable interference screw (BioRCI; Smith & Nephew) in antegrade fashion. The screw diameters were sized 1 mm above the graft/tunnel diameter and all screws were 30 mm long. Grafts were then dyed with methylene blue and speckled with white paint to allow for later digital image correlation (DIC) analysis. All constructs were created and tested by medical students under supervision of a sports medicine fellowship-trained orthopaedic surgeon and a doctoral laboratory manager.

Testing and Analysis

Biomechanical testing was carried out using an electromechanical load system consisting of a 6 degree of freedom hexapod (model R2000; Mikrolar, Hampton, NH) in series with a load cell (Theta IP65; ATI Industrial Automation, Apex, NC) mounted directly above the robotic platform. The load system was run in uniaxial mode and was operated in force control mode for the pretension and cyclical loading tests and motion control mode for failure tests. The load system had root means square error of <1% for the target versus actual load when operated under force control. For each construct, the proximal femoral cortical button was suspended over a secure metal plate attached directly to the load cell and the bone analogue was secured distally to the test platform with a clamp (Fig 2). Constructs were then pretensioned at 89 N for 10 minutes. After pretensioning, the grafts were cycled from 100 N to 250 N for 1,000 repetitions at 0.5 hertz before final load to failure testing at a rate of 0.5 mm per second. This load lies within the range of load on the ACL during weight-bearing and non−weight-bearing exercises (approximately 150-350 N). The difference in graft length at 100 N from the first 20 to 30 cycles compared with the last 10 cycles was used to represent cyclic displacement over time. Stiffness was calculated as the best-fit slope of the load displacement curve during load to failure. All trials were recorded on a high-definition camera to allow for later video analysis using GOM Correlate software (Trilion Quality Systems, Inc., King of Prussia, PA). DIC analysis of percentage change of length was performed to evaluate strain on each strand. The Student *t* test was used to assess statistical significance of the difference between the means of Groups 4S and 5S, with alpha level of 0.05. Analysis of variance and *t*-testing were used to evaluate individual strand lengthening on DIC. The primary outcome was cyclic displacement over time; at least 10 models were needed per group to detect a 10% difference in displacement with a beta level of 0.80. Other outcome measures of interest were stiffness and load to failure.

Table 1. Testing Results for the 4- and 5-Strand Semitendinosus and Gracilis Allograft Constructs

Parameter	4-Strand (n = 10)	5-Strand (n = 10)	P Value
Δ cyclic displacement at 100 N, mm*	0.87 ± 0.24	1.11 ± 0.24	**.038**
Δ cyclic displacement at 250 N, mm*	0.83 ± 0.24	1.07 ± 0.23	**.037**
Yield load, N	762 ± 151	707 ± 107	.35
Ultimate tensile strength, N	778 ± 155	735 ± 116	.49
Stiffness, N/mm	138 ± 5	138 ± 7	.96
Graft diameter, mm	8.5 ± 0.5	8.8 ± 0.6	.26

NOTE. Data are presented as mean ± standard deviation. Statistically significant differences are in bold.

*More than 1,000 repetitions, the difference in graft length between the first 20-30 cycles and the last 10 cycles.

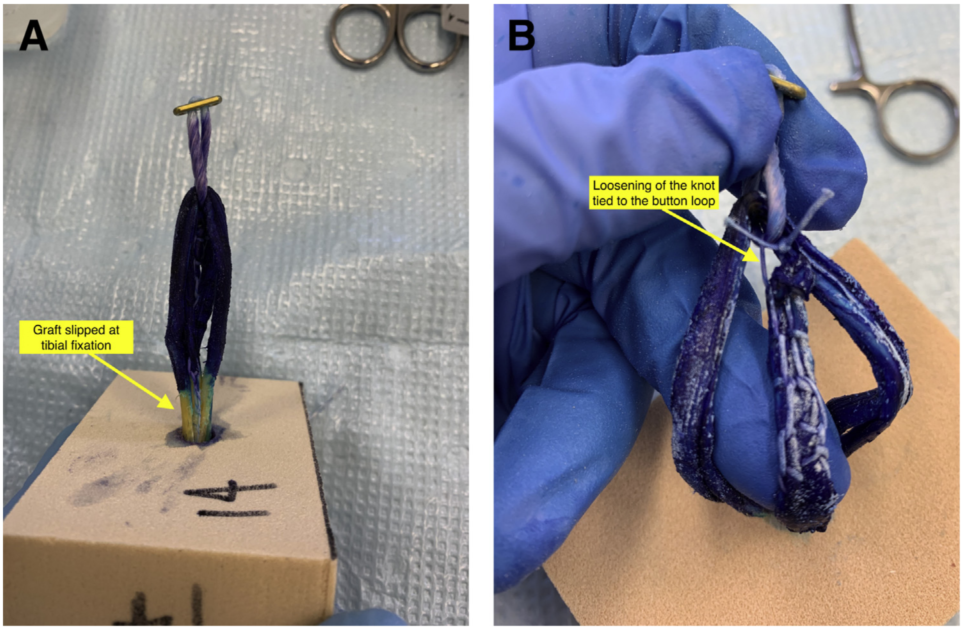

Fig 3. Methods of construct failure for the 4- and 5-strand anterior cruciate ligament grafts. On load to failure testing, the grafts most often failed at the interference screw (A). One 5-strand sample had obvious loosening at the tied knot at the suspensory loop (B).

Results

Cyclic displacement over 1,000 cycles was significantly lower in the 4S compared with the 5S group (0.87 vs 1.11 mm, $P = .038$, Table 1). There was no difference in load to failure or stiffness between constructs. Mean graft diameter was also similar between constructs at 8.5 to 8.8 mm. For tests in the 4S group, the visualized mechanism of failure was slippage at the screw (Fig 3A). In the 5S group, one test failed by obvious isolated fifth strand failure at the knot (Fig 3B), whereas in another test the cortical button snapped. We did not exclude any of the trials from analysis.

When reviewing high-definition video of each 5S trial, it was easily seen that on final load to failure testing, the tied fifth strand did not begin to stretch until the other 4 strands were stretched, indicative of obvious elongation at the fifth strand. DIC analysis (Fig 4) was performed to confirm these findings. Analysis of variance analysis of the 4S constructs revealed no difference in percentage change of length among the 4 strands ($P = .35$), whereas for the 5S construct, there was a trend toward significance ($P = .07$). On further analysis with comparison of just the fifth strand to the other 4 strands, the percentage change in length was significantly greater for the fifth strand (6.1 ± 3.4% vs 3.9 ± 2.5%, $P = .032$).

Discussion

Contrary to our hypothesis, the addition of a fifth strand for hamstring ACLR resulted in poorer biomechanical performance compared with a standard 4-strand graft. In particular, there was a statistically significant 20% increase in cyclic displacement for the 5S versus 4S group. Video analysis revealed a near-double increase in percentage change of lengthening in the fifth strand compared with the other 4 strands. Whether this amount of displacement is clinically significant remains to be determined.

Other biomechanical studies have compared tripled grafts with doubled grafts. Snow et al.[16] used bovine flexor tendons to compare a doubled tendon with a tripled tendon sewn to a cortical button using 1 different high-strength sutures, with interference screw tibial fixation. Similar to our study, authors found similar load to failure among grafts, but a trend toward increased displacement in the third (tied) tendon limb. Geethan et al.[14] also compared doubled with tripled bovine tendon grafts with different suture

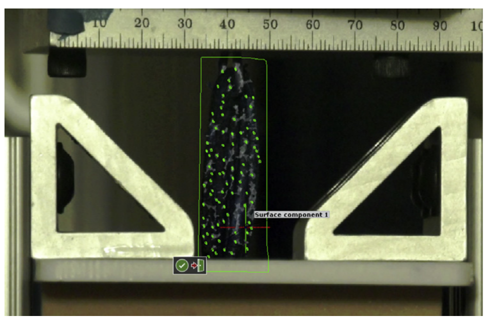

Fig 4. Example of still photograph from digital image correlation (DIC) video of the anterior cruciate ligament graft during biomechanical testing in the robot. The DIC software created fixed points on each strand (aided by white speckling on the dyed grafts) to calculate percentage change of length over time.

configurations (using cortical buttons and clamping the tibial end) and found significantly increased displacement of the tripled graft compared to the doubled graft only if the third limb was not sewn to the other limbs. Vaillant et al.[15] harvested cadaveric human tendons to create 4- and 5-strand constructs, suspended from cortical buttons with clamped tibial ends. The authors sewed together the strands for both constructs in addition to securing them to the button. Despite a significantly greater construct diameter in the 5-strand group, the authors found no difference in construct displacement or stiffness. Broadhead et al.[13] similarly found no biomechanical differences between 4- and 5-strand constructs using a sheep model, suspended from the button and clamped distally. In most of these studies, graft failure occurred either at the button, button loop, or tendon rupture at the distal clamp, as the majority did not use tibial interference fixation.

Compared with the aforementioned models, our study combined both 4- and 5-strand human allograft tissue with standard tibial interference fixation. By thus more accurately representing the in vivo scenario, we were able to show that graft failure most often occurred at the tibial fixation, which is in keeping with previous standard 4-strand biomechanical models,[17,18] and that this failure occurred before any catastrophic failure at the button. It is possible that if we had sutured the fifth strand to the other strands as in some of the studies listed previously, the 5S graft may not have displaced as much as it did. This is supported by the finding that the percentage change in length happened most notably at the tied fifth strand. In addition, incorporating suture tape along the graft could minimize elongation of the graft strands, as has been shown in several studies.[19]

In our study, the 4S and 5S groups had similar graft diameters. Both were on average greater than 8 mm. Biomechanical studies have shown that smaller diameter grafts do have decreased tensile strength.[20] However, the load to failure of these smaller grafts is still much greater than the load required for pull out at the interference screw. In clinical studies of patients younger than 20 years of age, use of hamstring autografts 8 mm in diameter or less has been found to be independently associated with increased risk of revision.[3] But not all studies have found this to be true.[6,21] It would be interesting to see if, had our 4S and 5S groups had statistically different diameters, whether this would balance the increased displacement that we found in the 5S group.

Limitations

As a controlled laboratory study, our findings do not perfectly represent the in vivo scenario. We have a few notable methodologic flaws. First, we did not use a tensioner system when placing the fixation of the interference screw at the tibial block. This was performed by hand, similar to how many surgeons place tibial interference in the operating room. To standardize constructs, we pretensioned all grafts on the robot prior to cyclical testing. However, there may have been slight variations in tension between the strands on initial screw placement. Second, we did not mandate a set length for grafts between the button and the interference screw. Thus, certain grafts were slightly longer than others. We based DIC calculations on percentage of change, but our measurements of cyclic displacement may be impacted by the relative increased length of certain graft constructs. Third, we did not experience a statistically different change in diameter when adding a fifth strand, which was contrary to what is often the clinical situation. This may be due to our small sample size, and potentially by chance the tendons used in the 5S group were smaller. However, we would not assume the intrinsic properties of the tendons to differ and thus we assume the relative lengthening of the 5S group over the 4S group to be due to the technique of the construct design (namely, the tied fifth strand). And, finally, we calculated that there was increased displacement of the 5S relative the 4S group, and that on video analysis there was greater percentage change in length on the tied fifth strand, our software did not have the ability to determine exactly where on the strand the lengthening was occurring (whether it was at the tied portion or at the tibial screw). This is an area of potential further exploration.

Conclusions

Compared with cyclically loaded 4S hamstring grafts, the 5S grafts had significantly increased displacement over time in a model of femoral suspensory and tibial interference screw fixation.

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