Original Article

Five-Strand Hamstring Grafts are Biomechanically Comparable to Four-Strand Grafts and Offer Greater Diameter for Anterior Cruciate Ligament Reconstruction

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Purpose: The purpose of this study was to compare the biomechanics of 4-strand and 5-strand hamstring constructs for anterior cruciate ligament grafts. **Methods:** Thirty-six human cadaveric hamstring grafts were tested in 3 different conditions: (1) graft femoral fixation complex, (2) graft femoral and tibial fixation (GFTF) complex using a human model, and (3) GFTF complex using a porcine model. Grafts were tested on a tensile testing machine. Four-stranded grafts served as the control group, and 5-stranded grafts served as the experimental group. Cyclic elongation, ultimate load to failure, stiffness, and diameter of the grafts were analyzed. **Results:** Average 4-strand graft diameter was 7.96 mm compared to 9.32 mm for the 5-strand graft ($P = .00017$). Average stiffness of grafts ≥8 mm was 105.04 N/mm compared to 85.05 N/mm for grafts <8 mm ($P = .04988$). There was a positive correlation between graft diameter and stiffness (13.4 N/mm per every 1 mm increase in diameter, r^2 value of 13.1%, and F-significance of 0.02778). There were no significant differences in terms of ultimate load to failure, cyclic elongation, or stiffness between the experimental groups. **Conclusion:** Five-strand hamstring grafts offer greater diameter and are biomechanically comparable to 4-strand equivalents at time 0. Grafts >8 mm offer significantly greater stiffness compared to grafts sized <8 mm. There is a weak positive correlation between graft diameter and stiffness. **Clinical Relevance:** A potential drawback to hamstring grafts is their variability in size. Five-strand hamstring grafts provide increased diameter in comparison to 4-strand equivalents and might be used when quadrupled graft diameter is <8 mm.

Anterior cruciate ligament reconstruction (ACL-R) is performed frequently in the United States, with more than 250,000 reconstructions annually.[1] Quadrupled semitendinosus (ST) and gracilis (G) tendon graft with suspensory femoral fixation and interference screw tibial fixation is a popular graft selection for ACL-R.[2] Although the suspensory femoral fixation serves as the gold standard in hamstring graft ACL-R, the best construct for tibial fixation is unclear. Interference screw tibial fixation demonstrates lower cyclic displacement and higher pullout stiffness in comparison to the other fixation methods.[3] A potential drawback of hamstring grafts is variability in size. Clinically, the use of smaller grafts has been found to increase the risk of failure.[4,5] Conversely, an increase in graft diameter will theoretically lead to improved patient-reported outcomes.[6] For example, a 2.0 mm—diameter increase in a 4-strand graft was found to correlate with a measurable increase on the Knee injury and Osteoarthritis Outcome Score.[6] Furthermore, this increase was either comparable to or greater than the minimal clinically important difference in International Knee Documentation Committee scores.[6] Methods to increase the size of a hamstring graft

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during surgery include allograft augmentation or conversion to a 5-strand (tripled ST tendon) graft. Tripling one of the hamstring grafts—generally the larger ST tendon—to create a 5-strand graft has been described and appears to be a viable alternative to allograft use.[7,8] Five-stranded grafts theoretically offer greater strength and increased tissue scaffold for subsequent ligamentization of the graft. However, the effect of increasing graft size by converting from 4 to 5 strands on the mechanics of tibial and femoral graft fixation is unknown. Tibial fixation is believed to be the factor limiting the mechanical properties of the entire construct at time 0. The purpose of this study was to compare the biomechanics of 4-strand and 5-strand hamstring constructs for ACL grafts. The null hypothesis was that there would be no difference in the ultimate load to failure, cyclic elongation, or stiffness between 4-strand and 5-strand ACL grafts at time zero.

Methods

Thirty-six pairs of human cadaveric hamstrings tendons (ST/G) were used for testing. All tendons were obtained from the Musculoskeletal Transplant Foundation (MTF Biologics, Edison, NJ) and tested according to the biosafety protocol from Rutgers Environmental Health & Safety. All samples were examined for any defects or signs of damage before be inclusion in the testing. The tendons were harvested from human cadavers (average age 51.7 ± 13.4 years old, 40% male, 60% female) and stored at −20°C until testing. On the day of testing, the tendons were thawed at room temperature, cleaned of excess fat, muscles, and fascia, and used for graft preparation. The bilateral pairs of harvested tendons (ST/G) from the same cadaver were used for the creation of 1 set of the tested grafts (4 and 5 strands) to provide a paired comparison. The 4-strand graft served as the control group, and the 5-strand graft served as the experimental group. Specimens were randomly distributed between testing groups in terms of laterality.

Graft Preparation

A pair of ST and G tendons from the same donor leg were used to create each graft. A nonabsorbable suture (no. 5 Ethibond; Ethicon, Somerville, NJ) was placed in whipstitch fashion at the free end of each tendon. The 4-strand graft was made by passing both the ST and G tendons through the continuous loop cortical button (EndoButton; Smith & Nephew, Andover MA) and folding them in half to create the graft with 4 equal length strands. To create the 5-strand graft, one end of the ST tendon was tied to the cortical button loop and secured with at least 5 squared knots. Subsequently, the tendon was passed through the extracortical button (ECB) loop and folded into thirds, with 2 folds creating 3 strands of equal length. The graft was augmented with the G tendon that was folded in half over the ST tendon to create 2 additional strands of equal length as described by Lavery et al.[7] The 5-strand graft consisted of 3 equal-length strands of ST tendon and 2 equal-length strands of G tendon.[7] The graft diameter was measured using a sizing tube (Smith & Nephew) as is done in the clinical setting. Finally, the grafts were manually pretensioned and attached to the tensile testing machine (5564 Instron Material Test Machine; Instron Corporation, Norwood, MA). Proximal fixation of each tendon was achieved by connecting the ECB to the custom-made jig fixed to the testing machine. Distal fixation differed depending on the testing model.

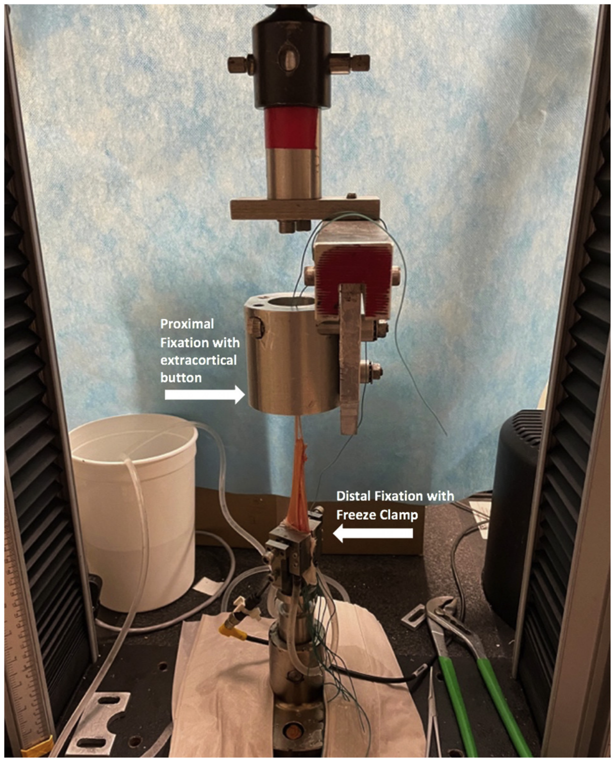

Fig 1. Graft with femoral fixation model.

Testing Model

Graft Femoral Fixation Model (Fem)

Grafts assigned to this testing condition were gripped distally with a freeze clamp (Electroforce; TA Instruments, New Castle, DE) that was attached to the testing machine (Fig 1). Six pairs of tendons were used for the Fem testing model. This model assessed the femoral fixation and tendon strength. The use of the freeze clamp eliminated the limiting effect of tibial fixation, which is known to be the weakest point of the entire construct. Proximally, the tendon was connected to the testing machine via the continuous loop cortical button device (EndoButton) secured in the custom-made jig attached to the Instron (5564 Instron Material Test Machine).

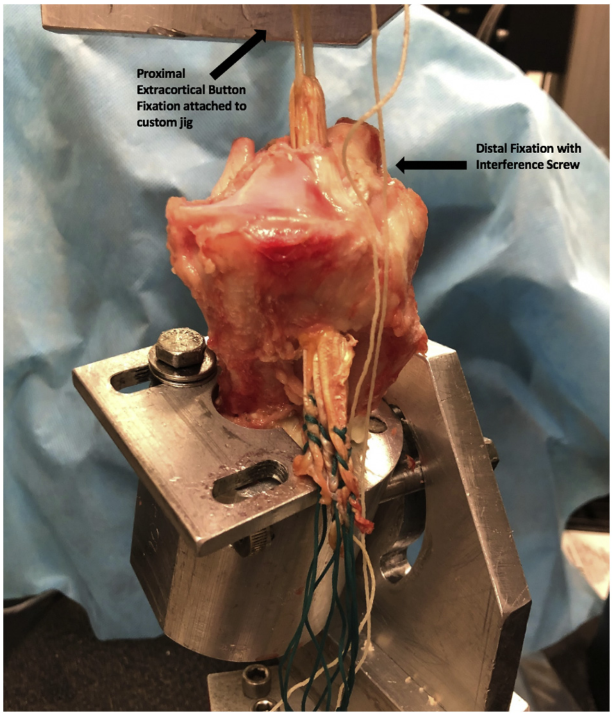

Fig 2. Graft with tibial fixation model.

Graft Tibial Fixation Human Model (Tib-H)

This condition replicated the widely applicable clinical method of distal fixation of the graft in the proximal tibia using an interference screw (Fig 2). Eight pairs of cadaveric tibias were obtained from the Musculoskeletal Transplant Foundation for testing (average age 51.7 $\pm$ 13.4 years old, 40% male, 60% female). Each tibia was inspected for any signs of damage or defect before testing. Tibias were cut at the level of approximately 15 cm from the tibial plateau. All soft tissue was removed from the bone. Each specimen was potted in a cylindrical mold using poly-methyl methacrylate cement (Frick Dental International Inc., Streamwood, IL) to a point 4 cm distal to the proximal aspect of the tibial tuberosity.[8] Using the Acufex Anatomic ACL Guide System (Smith & Nephew), a transtibial tunnel was created in each testing specimen. The diameter of each tunnel was equal to the measured diameter of the tested graft, with an average length of 39 $\pm$ 6.9 mm (35-50 mm range) long and a 55° inclination angle. Tunnels were drilled from the anteromedial cortex of the tibial metaphysis through the footprint of the ACL on the tibial plateau. The experimental ACL graft was pulled through the tibial tunnel until approximately 30 mm of the proximal end of the graft was outside the tunnel. The graft was manually tensioned on both ends and fixed into the tunnel via a titanium interference screw (Titanium RCI Screw; Smith & Nephew) inserted in a retrograde fashion. The screw selected for fixation was 25 mm long with a diameter equal to the diameter of the drilled bony tunnel. The tibia with inserted graft was loaded into the testing machine, and the proximal end of the graft was connected to the machine via the cortical button (EndoButton) secured in the custom-made jig. All fixation procedures were performed by the same orthopedic surgeon, avoiding inter-surgeon variation.

Graft Tibial Fixation Porcine Model (Tib-P)

Five pairs of porcine tibias, which were from 6 to 10 months of age, were obtained from a local slaughterhouse. All tibias were prepared according to the previously described protocol for the human cadaveric model (Tib-H). Randomly selected grafts were implanted and fixed to the tibias via titanium interference screws (Titanium RCI Screw). Tibias were loaded into the base of the Instron machine (5564 Instron Material Test Machine) and the cortical button loop was connected to the custom-made jig fixed to the testing machine proximally.

Biomechanical Testing

Biomechanical testing was done with a tensile testing machine (5564 Instron Material Test Machine). All samples were hydrated before testing and during the testing process using gauze pads soaked in phosphate-buffered saline solution. The experimental groups were subject to the same cyclic tension-relaxation. Graft constructs were preconditioned by cycling at 10 N to 50 N for 10 cycles, followed by 100 cycles from 50 N to 200 N, followed by pull-to-failure at a rate of 10 mm/min. Cyclic elongation, ultimate load, stiffness, and modes of failure were recorded. Mode of failure was defined as graft slippage, tendon rupture, failure of the suture connecting to continuous cortical button loop, or failure of the cortical button system itself.

Table 1. Graft With Femoral Fixation Model Biomechanics

Experimental Group	Count	Average Tensile Load $\pm$ 1 SD (N)	Average Cyclic Elongation $\pm$ 1 SD (mm)	Average Stiffness $\pm$ 1 SD (N/mm)
4-strand graft	6	1258.27 $\pm$ 173.28	1.24 $\pm$ 0.12	144.63 $\pm$ 26.20
5-strand graft	6	1227.63 $\pm$ 139.78	1.24 $\pm$ 0.27	158.04 $\pm$ 13.82
P value		.9945	.7430	.2935

SD, standard deviation.

Table 2. Graft With Femoral and Tib-H and Tib-P Model

Group	Count	Average Tensile Load ± 1 SD (N)	Average Cyclic Elongation ± 1 SD (mm)	Average Stiffness ± 1 SD (N/mm)
Biomechanics				
Tib-H (4-strand graft)	8	432.43 ± 118.95	3.83 ± 1.09	77.99 ± 20.01
Tib-H (5-strand graft)	8	471.37 ± 105.54	3.78 ± 2.20	77.35 ± 16.93
P value		.4999	.9562	.9457
Tib-P (4-strand graft)	5	455.64 ± 54.46	4.28 ± 3.33	69.59 ± 31.18
Tib-P (5-strand graft)	3	491.08 ± 159.50	3.07 ± 0.57	70.20 ± 17.02
P value		.7456	.4738	.9764
Comparison between Tib-H and Tib-P				
Tib-H	16	451.90 ± 110.47	3.81 ± 1.64	77.67 ± 17.91
Tib-P	8	468.93 ± 96.44	3.83 ± 2.61	69.82 ± 25.27
P value		.9826	.7147	.3867

SD, standard deviation; Tib-H, tibial fixation human; Tib-P, tibial fixation porcine.

Calculations and Statistical Analysis

Cyclic elongation was calculated as the displacement in gauge length between the end of the first cycle and the end of the one hundredth cycle. Ultimate load, stiffness, and measured diameter of the graft were analyzed. These parameters were compared using a 2-way analysis of variance followed by paired *t*-tests to determine statistical significance. A linear regression model was used to compare the association between graft diameter and stiffness. A 1-tailed *t*-test was used to compare stiffness between graft diameter groups. P values $<.05$ were considered significant. Statistical analysis was performed using Stata 16 software (StataCorp, College Station, TX) and Microsoft Excel (Microsoft, Redmond, WA). Based on our data, we could require 65 tests to obtain sufficient power to detect a difference between groups.

Results

Diameter by Graft Type

The average measured diameter of the four-strand graft was 7.96 mm ± 0.68 mm, compared to 9.32 mm ± 1.10 mm for the 5-strand graft ($P = .00017$).

Biomechanics

There were no significant differences in terms of ultimate load to failure, cyclic elongation, or stiffness within the experimental groups (Tables 1, 2).

Biomechanics between Tib-H and Tib-P

There were no statistical differences in terms of ultimate tensile load, cyclic elongation, stiffness, and graft diameter between human and porcine groups (Table 2).

Mode of Failure

There was a notable difference in the mode of failure between the testing groups (Table 3). The Tib-H group experienced eight failures due to graft slippage at the distal point of fixation, 2 failures of the proximal suture fixing the fifth strand to the cortical button loop, and 6 tendon ruptures. Within the femoral fixation group, there were 11 failures caused by ECB continuous loop failure and 1 tendon rupture. The Tib-P group experienced 7 graft slippages at the distal point of fixation and 1 tendon rupture. There were 2 catastrophic failures of the 5-strand Tib-P constructs, and these data were not included in analysis.

Stiffness between Graft Diameter Groups

All 3 testing groups (Fem, Tib-H, Tib-P) were analyzed comparing the relationship between graft diameter and stiffness using a 1-tailed *t*-test (Fig 3). The average stiffness of grafts sized ≥8 mm was 105.04 ± 44.27 N/mm compared to 85.05 ± 23.27 N/mm for grafts sized under 8 mm. These results were statistically significant ($P = .04988$).

Graft Diameter Versus Stiffness

The relationship between graft diameter and stiffness was measured using a linear regression model (Fig 4). There was a positive correlation between graft diameter and stiffness (stiffness increases by 13.4 N/mm for every 1 mm increase in graft diameter, r^2 value of 13.1% and F-significance of 0.02778).

Discussion

The results of our study confirmed that the five-strand graft offers a significant increase in diameter

Table 3. Mode of Failure

Fixation Method	Graft Slippage	Suture Failure	Tendon Rupture	ECB Failure
Tib-H (4 strand)	4	0	4	0
Tib-H (5 strand)	4	2	2	0
Fem (4 strand)	0	0	1	5
Fem (5 strand)	0	0	0	6
Tib-P (4 strand)	5	0	0	0
Tib-P (5 strand)	2	0	1	0

ECB, extracortical button; Fem, graft with femoral fixation; Tib-H, graft with femoral and tibial fixation human model; Tib-P, graft with femoral and tibial fixation porcine model.

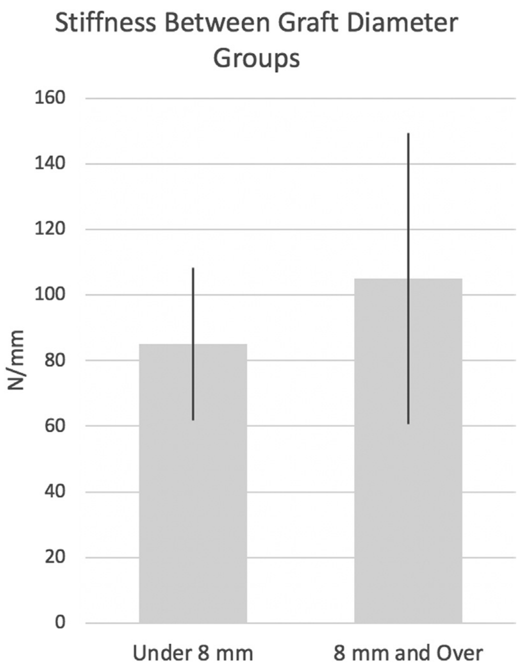

Fig 3. Stiffness between graft diameter groups.

relative to four-strand grafts. We also demonstrated that grafts sized 8 mm and over displayed significantly higher stiffness compared to grafts under 8 mm with a positive correlation between diameter and stiffness. However, there were no significant differences in stiffness, cyclic elongation, or ultimate load to failure between the 4- and 5-strand graft complexes tested in our study. A clinical and significant difference may exist but was not detected in our study, likely a result of the study being underpowered and possibility of a type II error.

Hamstring grafts are a popular choice for ACL-R. It has been reported that isolated quadrupled ST and G tendon grafts present superior biomechanics compared to the native ACL in terms of tensile strength and stiffness.[9,10] However, despite the biomechanical advantages, hamstring grafts have a high rate of failure, especially in the adolescent population.[11] This increased failure rate was previously investigated, and variability in the size and strength of fixation of the hamstring graft has been identified as contributing factors.[11]

Graft diameter has been proven to be an important factor for successful outcomes following ACL-R with hamstring grafts, as undersized grafts have been associated with higher rates of revision.[4-6,11,12] In a systematic review analyzing the failure rate of the hamstring grafts in relation to the graft diameter, Conte et al. concluded that four-strand hamstring grafts with a diameter of less than 8 mm were associated with significantly higher rates of failure compared to grafts greater than 8 mm in diameter with a relative risk of 6.8 (P = .008).[4] Additionally, Spragg et al. documented a 0.82 times lower likelihood of revision with every 0.5 mm incremental increase in graft diameter from 7.0 to 9.0 mm.[5]

Creating a 5-strand graft rather than a 4-strand graft has been shown to result in significantly increased graft diameter.[13-15] The results of our study confirmed that the five-strand graft created according to the technique proposed by Lavery et al.[7] offers a significant increase in diameter relative to four-strand grafts. Our 5-stranded constructs had an average measured diameter of 9.32 mm, compared to 7.96 mm for the 4-strand grafts (P = .00017). This resulted in a 17.09% increase in graft diameter and a 37.09% increase in cross-sectional area. Theoretically, the increase in graft diameter should have resulted in an increase in both tensile strength and stiffness. However, our results did not find a statistically significant increase in tensile strength. Boniello et al.[16] analyzed the relationship between hamstring graft

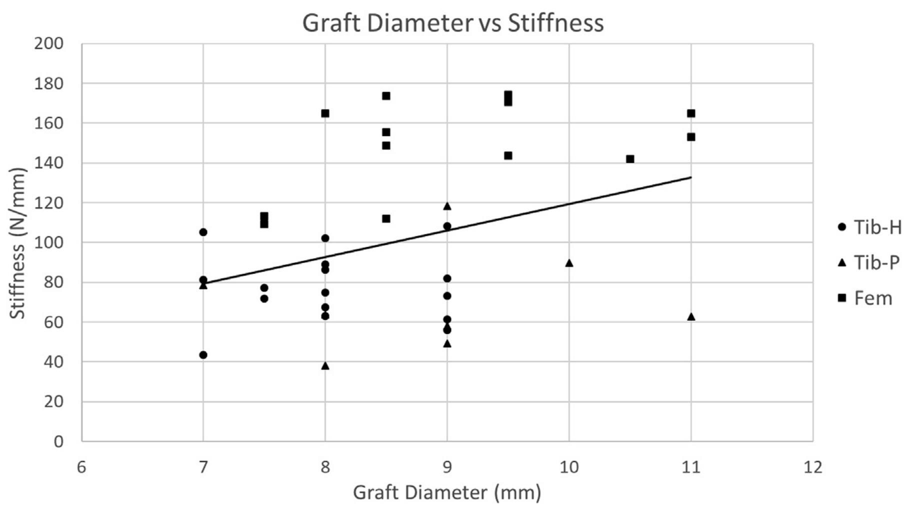

Fig 4. Correlation of graft diameter and stiffness. Graft with femoral fixation (Fem). Tib-H graft with femoral and tibial fixation human model (Tib-H). Graft with femoral and tibial fixation porcine model (Tib-P).

diameter and biomechanical strength and noted that a 1 mm increase in diameter from 8 to 9 mm was associated with a 12% increase in maximum tensile strength. However, that study tested the graft in isolation and did not account for the fixation technique. Of note, to achieve optimal tensile strength and stiffness of a hamstring graft, all strands must be equally tensioned during fixation.[9] Manual tensioning of the strands, as done in this study, makes achieving equal tension across all strands practically impossible.[9] This may explain why our results for the Fem, Tib-H, and Tib-P complexes showed no significant differences between the 4- and 5-strand grafts and may be a more accurate evaluation of the clinical setting.

In the current study, we aimed to describe the biomechanical differences between the fixation of 4- and 5-strand hamstring grafts tested in an environment mimicking the clinical setting. Biomechanical testing was performed on a human cadaveric model with the utilization of tibial interference screw fixation distally and suspension button proximally. Our study identified tibial fixation as the factor limiting the mechanical properties of the entire construct at time zero, with graft slippage from the tibial tunnel as a predominant mode of failure. To confirm that the results in the human cadaveric model were not influenced by the potentially poor bone quality of the cadaveric tibia, an alternate model using porcine tibias was tested. It was theorized that denser porcine bone might potentially offer improved tibial fixation of the graft, which could eliminate slippage as a mode of failure and theoretically allow for a greater load to failure.[17] A porcine model has been previously justified as an effective model for ACL reconstruction testing, and porcine tibias have been demonstrated to have increased bone density compared to human cadaveric tibias.[17-19] However, in our animal model, the presumed increased bone density of the porcine knees resulted in negligible improvement of fixation strength (3.77% increase in tensile strength [$P = .9$]), confirming that bone density was not a limiting factor in the human cadaveric model. Like the human cadaveric model, the porcine model showed a high failure rate caused by slippage in the tibial tunnel in both 4- and 5-strand groups.

There were no significant differences in stiffness or cyclic elongation between the 4- and 5-strand grafts tested as a Tib-H or Tib-P complex. Additionally, both tested groups were equivalent in terms of ultimate load to failure. These results were consistent with findings previously reported in the literature. Vaillant et al.[15] found that a 5-strand graft allowed for larger graft diameter without significantly changing mean stiffness or graft displacement at time 0. Calvo et al.[20] found no difference in clinical outcomes between 4-strand (>8 mm in diameter) and 5-strand grafts in terms of postoperative functional outcomes and rates of re-rupture at more than 2 years' follow-up. In our study, the lack of significant differences in biomechanics between 4- and 5-strand grafts resulted from failure of the tibial fixation.

To eliminate the limiting effect of tibial fixation, an additional testing condition (Fem complex) was added to the testing protocol. Instead of fixing the graft in the tibia, the tendons were gripped distally with the cryoclamp fixture. This model elicited important observations. It was found that the suturing of the ST tendon directly to the cortical button loop did not compromise the mechanics of the five-strand graft and allowed it to withstand a tensile load (1200 N) higher than the load created on the knee during normal everyday activities or typical postoperative rehabilitation protocol.[21] In all but 1 of the tested samples assigned to the Fem model, failure occurred on the continuous loop, and all grafts reached a tensile load (994 N) exceeding the reported load achieved in the human clinical setting model (452 N).[21] In the higher tensile condition of femoral fixation testing (Fem group), the 5-strand grafts showed no statistical difference in stiffness, ultimate tensile load to failure, or cyclic elongation compared to the 4-strand equivalent.

This suggests that an ideally constructed 5-strand graft has not only a greater diameter but is also biomechanically comparable. Furthermore, it suggests that the current technique for tibial fixation is the limiting factor in the biomechanical stability of the GFTF complex. However, these differences were revealed only in the high tensile load condition, which was not achievable in our clinical testing models. Improving the tibial graft fixation with a hybrid fixation technique, in combination with a thicker five-strand graft, may potentially reduce the failure rate of the construct in situations when a high load is applied to the graft. Graft survival relies heavily on the ability of the graft to resist the applied tensile load, which is limited by the fixation strength and graft stiffness. During postoperative rehabilitation, the forces applied on the ACL graft are typically low and do not exceed 400 N.[21] In this condition, both 4- and 5-strand grafts are sufficient to resist these forces, and consequently, the rate of graft survival is high. Occasionally, the peak force on the ACL, such as during single-leg landing from running to a stop, may significantly increase up to 1294 N.[22] Such forces can often be experienced during return to sport activities. In this situation, strong fixation and ability of the graft to resist the high tensile load may prevent a catastrophic failure.

Additionally, there may be further benefit to performing ACL reconstruction with a larger graft beyond the biomechanical properties of the graft at time zero. ACL grafts undergo progressive biological remodeling and neovascularization after reconstruction in a process of ligamentization.[23-26] This process includes cellular repopulation, vascularization, and synthesis of an extracellular matrix comprised of both large and small diameter collagen fibrils, as is seen in the native ACL, and results in tissue that resembles normal ACL both histologically and ultrastructurally.[23,24] Thus, although the 5-strand and 4-strand grafts may be biomechanically equivalent at time 0, the additional collagen scaffolding may increase ligamentization and result in increased graft longevity and resistance to lengthening or traumatic rupture.

Although our results did not achieve significance within the experimental groups, when all 3 testing groups were analyzed together, our results demonstrated that increased graft diameter was associated with a statistically significant increase in stiffness. Grafts with a diameter of 8 mm or larger were stiffer compared to grafts with diameters less than 8 mm ($P =$ .04988). Furthermore, our results showed a positive correlation between graft diameter and stiffness: a 1 mm increase in graft diameter was associated with a 13.4 N/mm increase in stiffness.

Future studies using different tibial fixation techniques, such as hybrid fixation, may help to further delineate the real biomechanical properties of hamstring grafts. Furthermore, long-term in vivo studies that measure clinical outcomes and collect tissue biopsies to assess ligamentization will further elucidate any differences between 5-strand and 4-strand hamstring graft constructs.

Limitations

There are several limitations to this study. The quality and density of the bones were unknown, which may influence the modes of failure for study groups using cadaveric knees. The cadaveric knee model also suffers from the inherent limitations of advanced cadaver age. Cadaver specimens are known to be higher in age than the average age of clinical ACL-R patients. The samples used in our study had an average age much higher than the average ACL-R patient. Secondly, although each graft construct was created to be reproducible, the variations in graft size, graft preparation, and suture technique may have influenced the results. The average measured diameter of the testing graft was 8.63 mm ± 1.12 mm, which was close to the 8 mm cut-off reported by Magnussen et al. that was found to be associated with higher rates of revision.[11] Next, the study was underpowered. Therefore, the null results demonstrated in the biomechanical differences between the 4- and 5-stranded grafts are susceptible to type II error. Finally, this was a time zero study using biomechanical outcomes. Therefore it is difficult to determine whether these results will correlate clinically or if a difference would emerge over time.

Conclusion

Five-strand hamstring grafts offer greater diameter and are biomechanically comparable to 4-strand equivalents at time 0. Grafts >8 mm offer significantly greater stiffness compared to grafts sized <8 mm. There is a weak positive correlation between graft diameter and stiffness.

Acknowledgments

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