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A Biomechanical Comparison of Allograft Tendons for Ligament Reconstruction

Jeremiah E. Palmer,* MD, Joseph P. Russell,† MS, Jason Grieshober,* MD, Abigail Iacangelo,† BS, Benjamin A. Ellison,† BS, T. Dylan Lease,† BS, Hyunchul Kim,† MS, R. Frank Henn III,* MD, and Adam H. Hsieh,*†‡ PhD
Investigation performed at the Fischell Department of Bioengineering, University of Maryland, College Park, College Park, Maryland, USA

Background: Allograft tendons are frequently used for ligament reconstruction about the knee, but they entail availability and cost challenges. The identification of other tissues that demonstrate equivalent performance to preferred tendons would improve limitations.

Hypothesis/Purpose: We compared the biomechanical properties of 4 soft tissue allograft tendons: tibialis anterior (TA), tibialis posterior (TP), peroneus longus (PL), and semitendinosus (ST). We hypothesized that allograft properties would be similar when standardized by the looped diameter.

Study Design: Controlled laboratory study.

Methods: This study consisted of 2 arms evaluating large and small looped-diameter grafts: experiment A consisted of TA, TP, and PL tendons (n = 47 each) with larger looped diameters of 9.0 to 9.5 mm, and experiment B consisted of TA, TP, PL, and ST tendons (n = 53 each) with smaller looped diameters of 7.0 to 7.5 mm. Each specimen underwent mechanical testing to measure the modulus of elasticity (E), ultimate tensile force (UTF), maximal elongation at failure, ultimate tensile stress (UTS), and ultimate tensile strain (UTε).

Results: Experiment A: No significant differences were noted among tendons for UTF, maximal elongation at failure, and UTε. UTS was significantly higher for the PL (54 MPa) compared with the TA (44 MPa) and TP (43 MPa) tendons. E was significantly higher for the PL (501 MPa) compared with the TP (416 MPa) tendons. Equivalence testing showed that the TP and PL tendon properties were equivalent or superior to those of the TA tendons for all outcomes. Experiment B: All groups exhibited a similar E. UTF was again highest in the PL tendons (2294 N) but was significantly different from only the ST tendons (1915 N). UTε was significantly higher for the ST (0.22) compared with the TA (0.19) and TP (0.19) tendons. Equivalence testing showed that the TA, TP, and PL tendon properties were equivalent or superior to those of the ST tendons.

Conclusion: Compared with TA tendons, TP and PL tendons of a given looped diameter exhibited noninferior initial biomechanical strength and stiffness characteristics. ST tendons were mostly similar to TA tendons but exhibited a significantly higher elongation/UTε and smaller cross-sectional area. For smaller looped-diameter grafts, all tissues were noninferior to ST tendons. In contrast to previous findings, PL tendons proved to be equally strong.

Clinical Relevance: The results of this study should encourage surgeons to use these soft tissue allografts interchangeably, which is important as the number of ligament reconstructions performed with allografts continues to rise.

Keywords: knee ligament reconstruction; allograft tendons; tibialis anterior; tibialis posterior; peroneus longus; semitendinosus

‡Address correspondence to Adam H. Hsieh, PhD, Fischell Department of Bioengineering, University of Maryland, College Park, Jeong H. Kim Engineering Building, Room 3242, College Park, MD 20742, USA (email: hsieh@umd.edu).

*Department of Orthopaedics, University of Maryland, Baltimore, Baltimore, Maryland, USA.

†Fischell Department of Bioengineering, University of Maryland, College Park, College Park, Maryland, USA.

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The anterior cruciate ligament (ACL) is the most commonly disrupted ligament in the knee, with an incidence of 100,000 to 200,000 injuries per year in the United States.[12] While most ACL reconstructions are performed using autografts, there is still a significant percentage (estimated at ~20%) performed using allograft tendons.[7] Recent meta-analyses have reported no significant differences in outcomes when comparing allograft and autograft tendons.[4,9,16,17,25] Several studies, however, have demonstrated a higher failure rate with allografts, suggesting that autografts should be preferred in younger, active patients.[3,24] Advantages of allografts include shorter operative times, availability of a variety of graft sizes, and, most significantly, lack of donor site morbidity.[6]

Historically, there have been several disadvantages to the use of allografts, including the risk of disease transmission, although sterilization and screening processes have all but eliminated this risk.[7,13] Graft availability and increased cost associated with allografts remain significant disadvantages. Numerous studies have demonstrated that even with the savings associated with decreased operative time, ACL reconstruction with an allograft remains more expensive than the harvest and use of autograft sources.[2,8,11,18] It is possible, however, that identifying additional adequate allograft donor sites could help minimize the problems of both availability and cost.

The aim of this study was to compare the biomechanical properties of 4 soft tissue allograft tendons: tibialis anterior (TA), tibialis posterior (TP), peroneus longus (PL), and semitendinosus (ST). We hypothesized that these allografts would exhibit similar characteristics when standardized by the looped diameter, allowing them to be used interchangeably for ligament reconstruction.

METHODS

An a priori power analysis was conducted to determine the number of specimens that would be needed for the study. The study consisted of 2 arms evaluating large and small looped-diameter grafts. Experiment A included TA, TP, and PL allograft tendons with looped diameters between 9.0 and 9.5 mm. Experiment B included TA, TP, PL, and ST allograft tendons with looped diameters between 7.0 and 7.5 mm. Historical data reported in the literature[10,19] were used to obtain expected SDs for the following outcome variables: ultimate tensile force (UTF; 590 N), ultimate tensile stress (UTS; 30 MPa), ultimate tensile strain (UTε; 0.04 mm/mm), and modulus of elasticity (E; 537 MPa). Practical differences to be detected were defined as what would be an acceptable window during normal ACL function for UTF (450 N), UTS (20 MPa), UTε (0.14 mm/mm), and E (300 MPa). Using a critical significance value of α = .05 and statistical power of $1 - \beta$ = .9, we conducted a power analysis according to Sokal and Rohlf[22] for each outcome variable to obtain average specimen numbers of n = 47 for experiment A and n = 53 for experiment B.

All tissues were donated by RTI Surgical and had undergone sterilization via BioCleanse (RTI Surgical) processing that does not include any radiation. We received all specimens without any identifying information attached. Initial looped diameters were measured using a standard pull-through gauge, similar to sizing techniques used in the operating room. Experiment A consisted of a total of 141 TA, TP, and PL allograft tendons (n = 47 each) with a looped diameter of 9.0 to 9.5 mm. Experiment B consisted of a total of 212 TA, TP, PL, and ST allograft tendons (n = 53 each) with a looped diameter of 7.0 to 7.5 mm. Before testing, the tendons were thawed to room temperature using 0.9% saline and were rehydrated for 30 minutes. Thickness, width, and gauge were measured using a digital caliper ($\pm$0.025 mm; Mitutoyo) on each strand of the looped tendon before testing. The cross-sectional area (CSA) of each strand was calculated from average thickness and width measurements obtained at 3 equally spaced intervals within the gauge length of the sample. A strand was assumed to be elliptical if the width-to-thickness ratio was $\leq$2.65 and was assumed to be rectangular if this ratio was higher than 2.65. The total CSA of each specimen equaled the sum of both strands.

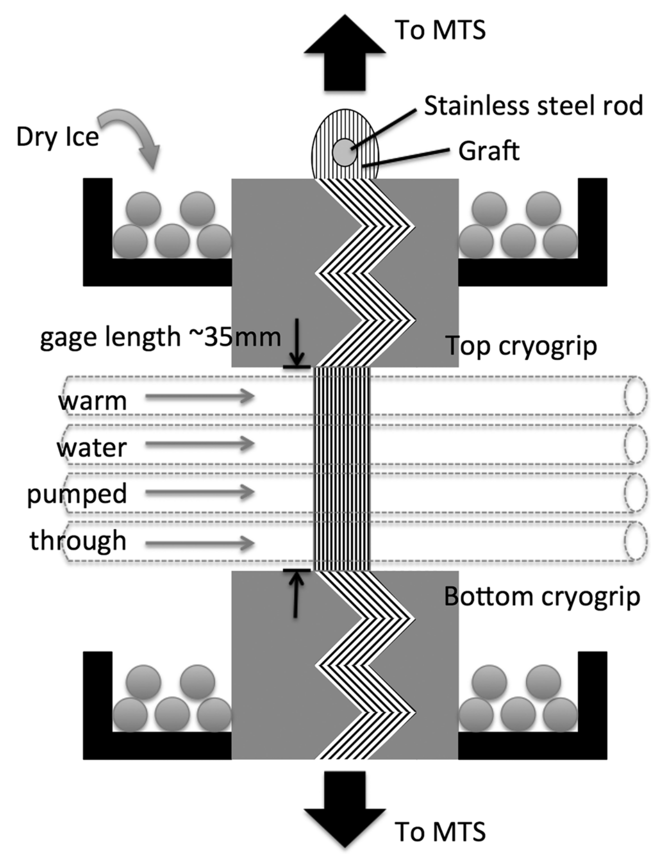

Figure 1. Schematic of the experimental setup. Two receptacles hold dry ice around the cryogrip fixtures. A series of tubes circulate warm water around the test length of the graft. MTS, materials testing system.

After thawing, hydration, and sizing, the specimens were placed into a cryogrip fixture that ensured the sample would not slip during testing and that each strand was equally tensioned. Each tendon was draped over a 1-cm stainless steel rod, such that the 2 cm of the ends of the tendon were clamped in the bottom cryogrip. The top cryogrip clamped the tissue immediately below the rod. Dry ice was then applied to freeze the cryogrips. A warm-water jacket surrounded the gauge length of each tendon to ensure that the specimen remained at body temperature (37°C) along the gauge length during testing (Figure 1). Gauge length was approximately 35 mm for each specimen. Each graft was mounted on a materials testing system (858 Mini Bionix II; MTS Systems), pretensioned, and tested using the following protocol: (1) 100-N hold for 60 seconds to remove any unnatural crimp, (2) loading at

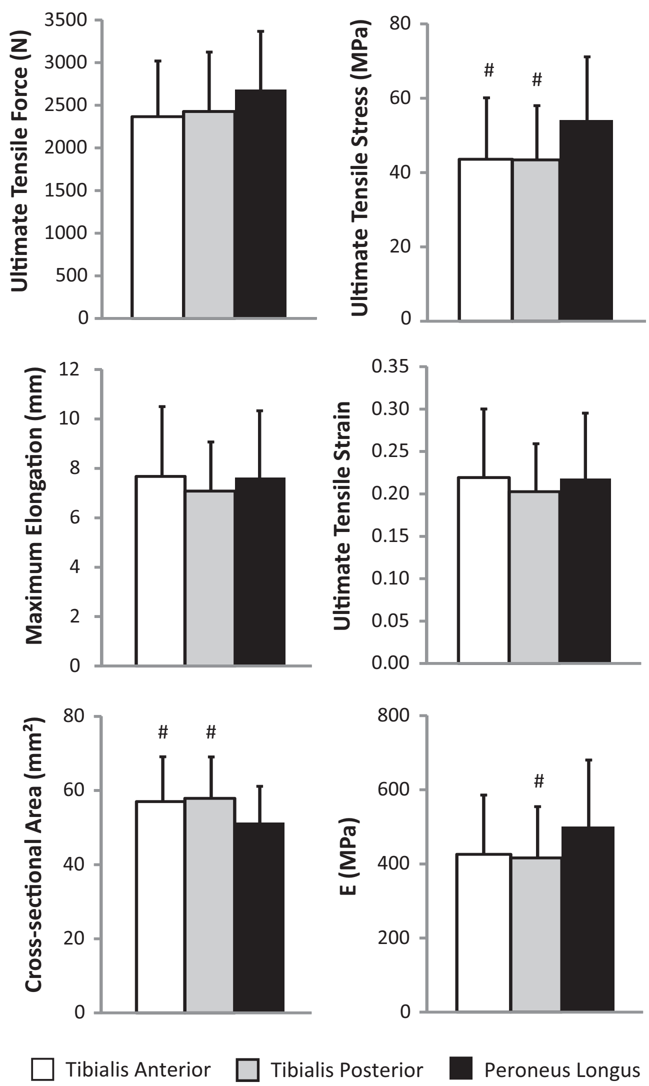

Figure 2. Comparison of biomechanical characteristics for tendons in experiment A (looped diameter, 9.0-9.5 mm). #Statistically significant difference compared with the peroneus longus group.

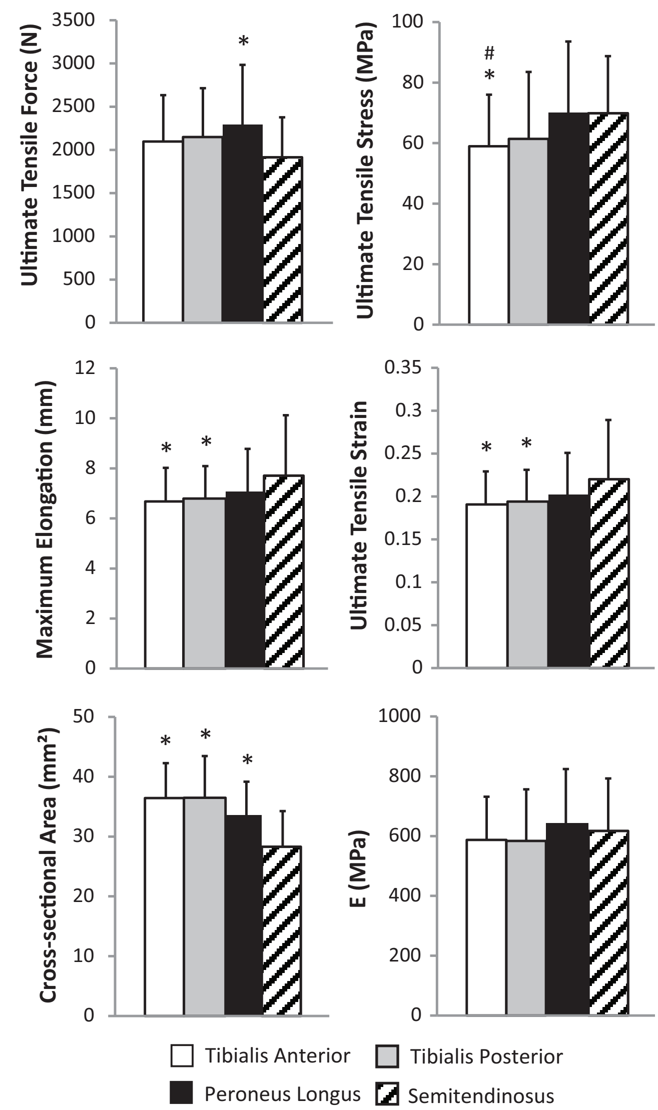

Figure 3. Comparison of biomechanical characteristics for tendons in experiment B (looped diameter, 7.0-7.5 mm). Statistically significant difference compared with the #peroneus longus and *semitendinosus groups.

2 mm/s up to 6% strain and hold for 100 seconds to simulate an athlete stretching, (3) unloading at 2 mm/s down to 3% strain to simulate the release of stretching, (4) loading at 2 mm/s up to 6% strain and hold for 100 seconds, (5) unloading at 50 N/s to <50 N to simulate relaxation, (6) cyclic loading at 1 Hz from 50 N to 250 N for 100 cycles to simulate running, and (7) loading at 35 mm/s until tensile failure to simulate a tendon rupture. UTF, the force measured at failure of the specimen, and maximal elongation at failure of the tissue were recorded. We then computed UTS, the CSA-normalized value of the force at failure, and UTε, the maximal elongation of the specimen normalized by initial length. E, the normalized metric of tissue stiffness, was obtained from the linear portion of the stress-strain curve, the region typically associated with physiological load bearing for ligaments/tendons.

Two statistical analyses were conducted. First, to determine whether differences between groups were statistically significant, we conducted 1-way analysis of variance with Tukey post hoc tests for multiple pairwise comparisons, using critical significance levels of $\alpha = .05$. Second, because we were interested in whether each tissue was suitable for ligament reconstruction, we conducted equivalence testing as described by Walker and Nowacki.[23] With equivalence testing, differences between each group and a reference group are computed. If the 90% CIs of these differences fall entirely within a predefined range (equivalence margin, $\pm\delta$), the 2 tissues are deemed equivalent. In this study, δ was 25% of each outcome measure because the coefficient of variation for properties (ie, dispersion of the data) was, on average, 27.8%. Using $\delta = 25\%$ provides a stringent

TABLE 1
Equivalence Testing Comparing TP, PL, and ST Tendons Against TA Tendons[a]

	Experiment A		Experiment B		
	TP vs TA	PL vs TA	TP vs TA	PL vs TA	ST vs TA
UTF, N					
$\mu_x - \mu_{TA}$	60	318	79	225	−155
90% CI	−156 to 276	105 to 532	−97 to 255	27 to 422	−314 to 5
δ (25% TA)	592	592	517	517	517
E, MPa					
$\mu_x - \mu_{TA}$	−10	75	−3	56	30
90% CI	−57 to 38	20 to 129[b]	−54 to 47	3 to 108	−21 to 82
δ (25% TA)	107	107	147	147	147
Elongation, mm					
$\mu_x - \mu_{TA}$	−0.59	−0.06	0.11	0.39	1.03
90% CI	−1.37 to 0.19	−0.94 to 0.83	−0.31 to 0.54	−0.10 to 0.88	0.40 to 1.65[c]
δ (25% TA)	1.92	1.92	1.67	1.67	1.67
UTS, MPa					
$\mu_x - \mu_{TA}$	0	11	2	11	11
90% CI	−5 to 5	5 to 16[b]	−4 to 9	5 to 18[b]	5 to 17[b]
δ (25% TA)	11	11	15	15	15
UTε, mm/mm					
$\mu_x - \mu_{TA}$	−0.017	−0.002	0.003	0.011	0.029
90% CI	−0.039 to 0.006	−0.027 to 0.024	−0.009 to 0.015	−0.003 to 0.025	0.012 to 0.047[c]
δ (25% TA)	0.055	0.055	0.048	0.048	0.048
CSA, mm^2					
$\mu_x - \mu_{TA}$	0.88	−5.64	0.07	−2.85	−8.14
90% CI	−2.84 to 4.60	−9.14 to −2.13	−1.99 to 2.12	−4.68 to −1.02	−10.02 to −6.25[d]
δ (25% TA)	14.25	14.25	9.11	9.11	9.11

[a]CSA, cross-sectional area; E, modulus of elasticity; PL, peroneus longus; ST, semitendinosus; TA, tibialis anterior; TP, tibialis posterior; UTε, ultimate tensile strain; UTF, ultimate tensile force; UTS, ultimate tensile stress; δ, equivalence margin (the range of values close enough to be considered equivalent); $\mu_x - \mu_{TA}$, difference between group tested and reference (TA group).
[b]90% CI superior to TA, [c]close to δ, and [d]inferior to TA; all others statistically equivalent.

threshold to determine whether another tissue can be considered to be equivalent for each property. Because TA and ST tendons are the preferred tissue sources for ACL reconstruction, we used these groups as the reference. Therefore, in experiment A, differences in averages between the TA group and each of the other tissues (TP, PL, ST) were calculated. For experiment B, differences against both the TA and ST groups were computed versus each of the other groups (TP and PL).

RESULTS

All tendons were maintained at approximately 37°C across the gauge length of the specimen, and each specimen ruptured within the gauge length during the ramp load to failure phase. In experiment A (Figure 2), we found no significant differences in any of the properties assessed between the TA and TP groups. For UTF, although measured values for the PL tendons were higher than for the TA and TP tendons, no significant differences were observed among the 3 groups. The PL tendons did possess an 11% to 13% smaller CSA compared with the TA and TP tendons, a difference that was statistically significant ($P < .05$). Hence, the PL tendons exhibited a UTS that was significantly higher (~20%) than the TA and TP groups ($P < .005$). E values for the PL tendons were significantly higher than those for the TP tendons ($P < .03$) and trended higher compared with the TA tendons ($P < .06$). UTε and maximal elongation at failure were similar among all 3 groups.

For experiment B (Figure 3), we found that the PL tendons again exhibited the highest UTF, although they were significantly different only from the ST group ($P < .005$). The ST group had the greatest UTε and was significantly higher than the TA and TP groups ($P < .05$), although not significantly different from the PL group. The CSA was also significantly smaller in the ST group compared with the TA, TP, and PL groups ($P < .001$). The TA group possessed a significantly lower UTS than both the PL and ST groups ($P < .05$). E was similar among all groups.

Equivalence testing to compare ligaments to TA tendons (Table 1) showed that the TP tendons were equivalent to the TA tendons for all measured properties in both experiments A and B. The PL tendons were equivalent to the TA tendons for most structural properties, the exceptions being E and UTS in experiment A and UTS in experiment B. It is worth noting, however, that although not equivalent, values of both E and UTS were superior to those of the TA tendons. For the ST tendons (experiment B only), equivalence testing showed that the CSA was inferior to the TA tendons and that UTS was superior to the TA tendons, consistent with the initial comparison of

TABLE 2
Equivalence Testing Comparing TA, TP, and PL Tendons Against ST Tendons[a]

	Experiment B		
	TA vs ST	TP vs ST	PL vs ST
UTF, N			
$\mu_x - \mu_{ST}$	155	234	379
90% CI	−5 to 314	69 to 398	192 to 566[b]
δ (25% ST)	479	479	479
E, MPa			
$\mu_x - \mu_{ST}$	−30	−34	25
90% CI	−82 to 21	−89 to 22	−32 to 82
δ (25% ST)	154	154	154
Elongation, mm			
$\mu_x - \mu_{ST}$	−1.03	−0.91	−0.64
90% CI	−1.65 to −0.40	−1.54 to −0.30	−1.31 to 0.03
δ (25% ST)	1.93	1.93	1.93
UTS, MPa			
$\mu_x - \mu_{ST}$	−11	−9	0
90% CI	−17 to −5	−15 to −2	−7 to 7
δ (25% ST)	18	18	18
UTε, mm/mm			
$\mu_x - \mu_{ST}$	−0.029	−0.026	−0.018
90% CI	−0.047 to −0.012	−0.044 to −0.008	−0.037 to 0.001
δ (25% ST)	0.055	0.055	0.055
CSA, mm^2			
$\mu_x - \mu_{ST}$	8.14	8.20	5.29
90% CI	6.25 to 10.02[b]	6.13 to 10.27[b]	3.44 to 7.13[b]
δ (25% ST)	7.07	7.07	7.07

[a]CSA, cross-sectional area; E, modulus of elasticity; PL, peroneus longus; ST, semitendinosus; TA, tibialis anterior; TP, tibialis posterior; UTε, ultimate tensile strain; UTF, ultimate tensile force; UTS, ultimate tensile stress; δ, equivalence margin (the range of values close enough to be considered equivalent); $\mu_x - \mu_{ST}$, difference between group tested and reference (ST group).

[b]90% CI superior to ST; all others statistically equivalent.

averages. However, it contrasted with previous comparisons in finding that UTε and elongation of the ST tendons were not inferior to the TA tendons.

In comparing ligaments to ST tendons (Table 2), equivalence testing showed that the TA, TP, and PL tendons were all superior to the ST tendons in CSA and that the PL tendons were superior to the ST tendons in UTF. All other measures were equivalent to the ST tendons.

DISCUSSION

This study presents important data comparing the biomechanical characteristics of several soft tissue allografts. The results demonstrate that the TP and PL tendons of a given looped diameter compare favorably with the TA tendons; the PL tendons were equally strong in contrast to previous reports; and in smaller diameter grafts, all allografts tested were at least equivalent to the ST tendons.

Historically, the TA has been the tendon of choice among soft tissue allografts, especially where larger (9.0-9.5 mm) grafts are needed.[1] Authors have noted that it has superior biomechanical properties,[6,21] and a randomized trial demonstrated no significant differences in clinical outcomes at 2-year follow-up when compared with soft tissue autografts.[15] Several studies have compared the biomechanical properties of various soft tissue allografts. Haut Donahue et al[14] compared fresh-frozen, single-loop TA and TP tendons with double-loop ST and gracilis (STG) grafts, using 16 samples per graft type, and found that tensile strength and stiffness were not significantly different from the STG graft. Both the TA and TP tendons were also stronger than the native ACL and a patellar tendon graft. Pearsall et al[20] examined the biomechanical properties of the TA and TP tendons and further included PL tendons. Again, TA and TP allografts demonstrated excellent UTF (3412 and 3391 N, respectively), exceeding those of the native ACL, bone-tendon-bone (BTB), and STG grafts. The PL tendons showed a lower UTF (2483 N), but all 3 grafts demonstrated stiffness exceeding historical reports of other graft types. With a similar experimental setup, Chan et al[5] compared fresh-frozen TA, TP, and PL tendons to BTB grafts, using 18 samples per graft type, and found that all 3 had similar ultimate tensile strength and stiffness values, which were significantly greater than those of BTB grafts. In all these cases, the relatively low sample sizes make it difficult to establish the statistical equivalence of the other types of allograft tissues with the TA tendon. In our study, the large number of specimens tested enabled us to use equivalence testing to demonstrate that TA, TP, PL, and ST tendons are mechanically

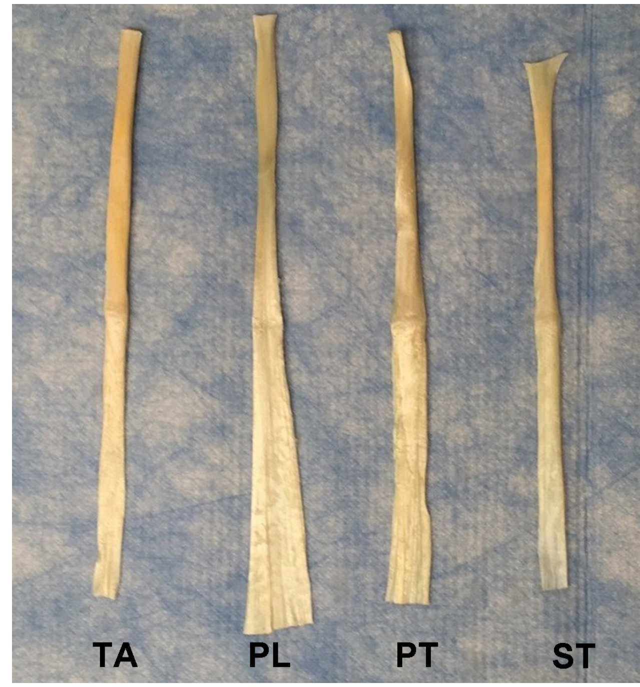

Figure 4. Photograph of representative samples demonstrating gross morphological characteristics of each tendon type. PL, peroneus longus; ST, semitendinosus; TA, tibialis anterior; TP, tibialis posterior.

noninferior in almost every metric that we benchmarked, although we did not test BTB grafts.

Comparing absolute values among these various biomechanical studies is difficult because differences in protocols affect outcomes. However, the current report builds on the experience of previous authors. Our data are comparable in that the TA and TP tendons appear to be biomechanically equivalent across all properties measured and comparable with the strength of the ACL and other graft sources as reported in the literature.[5] This supports the use of these tendons as adequate allografts in ligament reconstruction about the knee.

Additionally, the biomechanical characteristics of tendons at time zero from all groups compared favorably with historical values for the native ACL. Noyes et al[19] studied the mechanical properties of the ACL from young adults and found that the maximum load to failure averaged 1725 N. Woo et al[26] studied the healthy ACL from young adults but evaluated the entire femur-ACL-tibia complex in an anatomic orientation and concluded that the true ultimate load to failure was likely higher at 2160 N.

Compared with previous measurements of allograft tendon biomechanical tests, however, some differences were notable. For instance, we measured lower values for E and UTS than Haut Donahue et al.[14] The distinctions could potentially be attributable to our extensive preconditioning protocols and the proprietary BioCleanse sterilization process that involves both mechanical and chemical agitation. Interestingly, our data regarding PL tendons were discordant with previous reports. First, despite having the same looped diameter as measured using a pull-through gauge, PL tendons had a significantly smaller measured CSA. This may have been because of the fan-like shape of the PL grafts as opposed to the more uniformly cylindrical TA and TP grafts (Figure 4). Despite this, PL grafts were not significantly different regarding UTF or UTε and demonstrated a greater UTS than both the TA and TP tendons and a greater E compared with TP tendons. This is in contrast to the findings of both Pearsall et al[20] and Chan et al,[5] who found the TA and TP tendons to be stronger than the PL tendons. We believe that this is likely because in both investigations, the tendons were donor matched, whereas we matched all tendons by the looped diameter. Because an average donor's TA and TP tendons are larger than the PL tendon, this could confound those analyses. In the current study, PL tendons were at least equally strong and stiff when compared with TA and TP tendons, suggesting that they too can be used interchangeably given a looped diameter.

In the smaller diameter grafts of experiment B, PL tendons were similar to TA and TP tendons but clearly superior to ST tendons, with a significantly greater load to failure. The ST group was also inferior to both the TA and TP groups regarding strain and maximal elongation. However, the ST group also demonstrated a significantly smaller CSA. The smaller size likely accounts for differences in biomechanical characteristics and limits our ability to test our hypothesis regarding the ST grafts. It is also interesting to note that the normalized values of UTS, UTε, and E were different between the same tissues from experiments A and B. Although we do not fully understand the basis for these distinctions, they were consistent across the experiments. We speculate that differences might be associated with systematic errors in approximating CSAs or possibly genetic and epigenetic differences in tissue architectures associated with smaller and larger donor tissues.

The current study differs from previous reports in several ways. First, we conducted an a priori analysis to determine the necessary sample size to detect practical differences among the groups. This resulted in a sample size far greater than that of any previous biomechanical study comparing soft tissue allografts.[5,14,20] Second, the specimens tested were fully prepared grafts, having undergone sterilization and processing, more closely representing a clinical scenario. In addition, because our goal was to obtain a clinically relevant comparison of allograft tissues, our experimental design involved grouping specimens according to the looped diameter rather than strictly measured CSAs. Although this could contribute to greater variability, we think that this approach did not detract from our findings because SDs of our measurements were comparable with those reported in the literature.

This study does have limitations common to all in vitro biomechanical studies. Testing assessed the initial biomechanical characteristics of each tendon type, and we were unable to assess important clinical aspects such as pull-out strength with fixation, graft incorporation, graft function over time, or clinical function in vivo.

In conclusion, the results of this study indicate that, compared with TA allografts, TP and PL tendons of a given looped diameter exhibit noninferior (ie, equivalent or superior) initial biomechanical strength and stiffness characteristics at the time of surgery. ST tendons were mostly similar to the TA tendons but exhibited a significantly higher elongation/UTε and smaller CSA. For smaller looped-diameter grafts, all tissues were noninferior to the ST tendons. In contrast to previous findings, PL tendons proved to be equally strong. This should encourage surgeons to use these soft tissue allografts interchangeably, which is important as the number of ligament reconstructions performed with allografts continues to rise.

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