Preliminary Mechanical and Ultrastructural Characterization of Pediatric Anterior Cruciate Ligaments and Tendons Used for Reconstruction

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Introduction

A large amount of cadaveric research has been devoted to the biomechanical characterization of knee tendons and ligaments in adults; however, relatively little is known about the pediatric population due to the rarity of these specimens. Extrapolating data back to pre-pubescent ages is inadequate, and the clinical need for this data has grown along with the recent increase in diagnosed anterior cruciate ligament (ACL) tears in skeletally immature patients.[1] Ideally, surrogate grafts should closely parallel the native pediatric ACL in both its biologic properties and mechanical durability. Thorough characterization of the ACL and potential autograft options will help to further improve surgical outcomes in pediatric patients. The purpose of this study was to characterize the mechanical properties and ultrastructure of ACLs and the most common tendons used for reconstruction in the pediatric knee. Our goal was to gain a better understanding of the structure and function of these tissues to improve surgical outcomes.

Methods

Mechanical Testing

Five fresh-frozen knee specimens from separate donors were used in this study; three male, two female specimens with average age of 9.2 years. ACLs, patellar ligaments, quadriceps tendons, and semitendinosus tendons were fine dissected free from the knee and subsequently cut into dog-bone shapes at the mid substance (ACL, patellar ligament) or distal third substance (quadriceps and semitendinosus tendon) with a custom-built jig. Cross-sectional areas of the prepared specimens were measured with a noncontact laser-based measurement system.[2] Specimen ends were placed in custom aluminum clamps and attached to a 4500N load cell on a universal testing frame (TA Instruments ElectroForce 3550, Eden Prairie, MN) to perform uniaxial tensile testing. Specimens were subjected to a standard preload, cyclic preconditioning, and stress-relaxation protocol before a ramp to failure at a constant quasistatic strain rate of 0.03% per second. Ultimate strain and ultimate stress were recorded while Young's modulus and stiffness were calculated as the slope of the stress-strain curve and load-displacement curve, respectively. Strain energy density was calculated as the area under the stress-strain curve.

Histology and Transmission Electron Microscopy

A patellar tendon from the contralateral knee of a specimen (age 9, F) was selected as a pilot sample for histology and transmission electron microscopy (TEM) analysis. The specimen was fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin & eosin (H&E) for viewing under a standard light microscope. The TEM sample was fixed, embedded in resin, cut orthogonal to the axis, and stained with uranyl acetate for examination with a JEOL 1010 electron microscope. Ten micrographs were obtained at 60,000x magnification for each specimen. Micrographs were analyzed using a semi-automated threshold and segmentation protocol in Image-J/Fiji software (NIH, Bethesda, Maryland). Cross-sectional areas of collagen fibrils was determined using the minor fibril diameter and the distribution of fibril areas was fitted using a kernel density estimation (Figure 2C).

Results

Mechanical properties for the pediatric ACLs and anterior cruciate ligament reconstruction (ACLR) candidate grafts are summarized in Table 1 and Figure 1. The patellar ligament exhibited mechanical properties that were most similar to that of the ACL, particularly for ultimate stress, ultimate strain, Young's modulus, and strain energy density. The same structural properties in the adult populations seem to exist in the pediatric condition, despite considerably weaker mechanical properties. This was expected based on previous studies in immature animal models.[3,4] In adults, hamstrings tendons have previously been shown to exhibit significantly higher elastic modulus (1036±312 MPa) and ultimate stress values (120.1±30.0 MPa) than other graft candidates, including the patellar ligament (417±107 MPa, 76.2±25.1 MPa).[5]

Table 1. Mechanical Properties of Pediatric ACLs and Tendons used for Reconstruction

Tissue	n	Age Group	Ultimate Stress (MPa)	Ultimate Strain(%)	Young's Modulus (MPa)	Stiffness (N/mm)	Strain Energy Density (MPa)
ACL	4	9-11	5.24 (2.20)	46.43 (5.71)	24.32 (15.63)	40.48 (18.83)	0.84 (0.43)
Patellar Ligament	5	7-11	5.23 (3.07)	44.58 (8.38)	27.00 (6.86)	20.25 (5.34)	1.17 (0.86)
Quadriceps Tendon	5	7-11	12.09 (8.26)	51.02 (21.91)	64.61 (68.78)	42.71 (35.84)	2.54 (1.91)
Semitendinosus Tendon	5	7-11	27.87 (12.88)	31.5 (9.17)	194.34 (28.54)	98.81 (15.57)	3.05 (1.90)

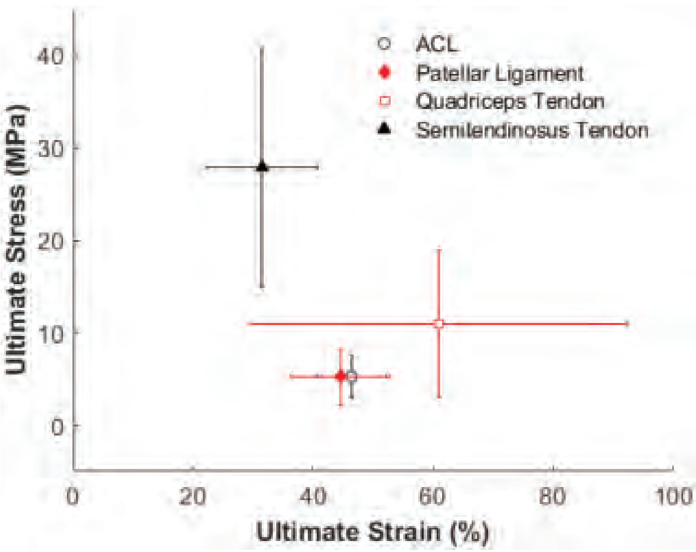

Figure 1. Ultimate stress-strain plot for the ACL, patellar ligament, quadriceps tendon, and semitendinosus tendon. Points represent the average ultimate stress and ultimate strain and the error bars indicate the standard deviation.

Histology of the single patellar ligament tested appeared to have the typical characteristics of a healthy tendon: organized and anisotropic in structure with collagen fibers closely packed along the fascicle's longitudinal axis (Figure 2A). Fibroblasts were located within and between the fascicles, in parallel with the collagen fibers. The characteristic crimp patterning of the collagen fibers was also visible. Approximately 5,000 fibrils were analyzed over 10 TEM micrographs of the patellar tendon (Figure 2B). Average fibril area fraction was 61.6±2.6% and average fibril concentration was 67±7/µm^2. Fibril areas were bimodally distributed between 508.6 nm^2 and 27,442.0 nm^2, with smaller area fibrils exhibiting higher frequency (57.4%) than larger area fibrils (42.6%) (Figure 2C).

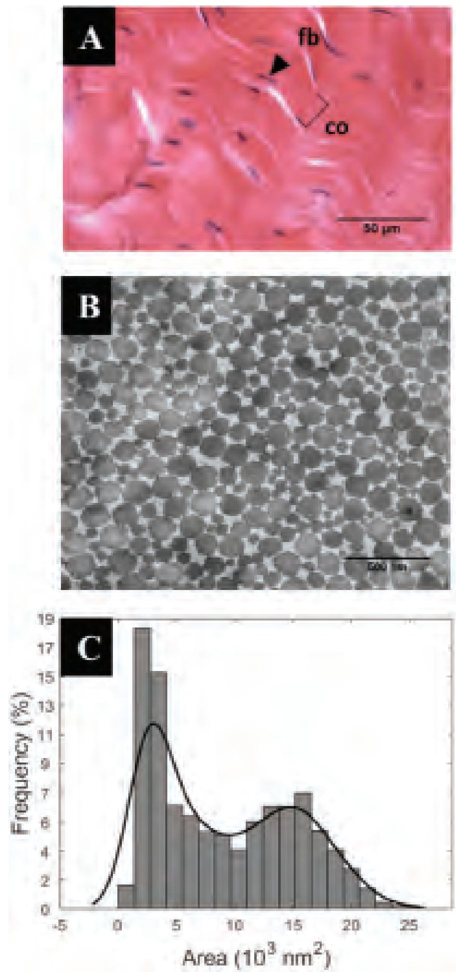

Figure 2. Representative histologic staining for a longitudinal section of patellar tendon **(A)**, showing fibroblasts (fb) and collagen fibers (co) (H&E, original magnification 300x) and representative TEM micrograph for a cross section of patellar tendon **(B)** (original magnification 60,000x). The distribution of collagen fibril areas over 10 separate micrographs was fitted with a kernel density estimation **(C)**.

Discussion

The methods and results from different studies on the mechanical properties of adult knee tendons and ligaments vary markedly, making comparisons difficult. This is in agreement with our data for a pediatric population, which showed that the semitendinosus tendons are stronger and less compliant than the quadriceps or patellar tendons. The histomorphometry of the pediatric patellar ligament did not appear qualitatively different than what has been documented in the literature for healthy, adult humans.[6,7] Previous studies conducted with TEM have reported collagen fibril area fractions and densities from adult cadaveric patellar tendon specimens that are comparable to ours.[7,8]

Based on the similarities of their material properties alone, the patellar ligament may be the better graft candidate for pediatric ACLR. However, it is important to take into consideration that the mechanical properties of ACLR grafts have been demonstrated to decrease during the remodeling process in adults and never return to normal.[9] Therefore, it may be more appropriate to utilize a hamstrings tendon graft, which is significantly stronger than the patellar ligament and could resist the loads that make the ACL prone to reinjury or caused the injury in the first place.

Conclusion

More work is needed to explore the full implications of this preliminary study. We intend to complete a comprehensive ultrastructural examination for the ACL, quadriceps tendon, and semitendinosus tendon, including examination with polarized light microscopy. This suite of data can be used to inform the design and selection of grafts for reconstruction and also to develop constitutive computational models that can be applied to clinically relevant loading conditions that are difficult to test with bench-top experiments alone.

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