4. Mechanical Characteristics of Porcine Patellar Tendons

4.1 Introduction

The prevalent surgical treatment for a ruptured anterior cruciate ligament (ACL) is reconstruction of the injured tissue with a substitute graft. Ideally, the graft should exhibit similar mechanical properties and geometry as the intact ACL to avoid laxity and abnormal knee kinematics [26]. In practice, a central third bone-patellar tendon (PT)-bone graft is often selected. Researchers have shown the mechanical properties of PT to be initially greater than those of the ACL [11,15,22]. Immediately after implantation, the properties of the graft decrease to a level below those of the ACL, and the long-term prognosis is less than optimal [5,6,9,10,13,24]. Further research into this reconstruction procedure is necessary to improve the outcome.

A number of animal models have been used to examine ACL reconstruction procedures, including canine [5,10,24,33], goat [18,19,21], primate [9,13], rabbit [2,3,6], and sheep [1,25,35]. Recent studies have suggested the porcine knee is the animal model that best represents forces developed in the human ACL [31]. Hence, the porcine model may be appropriate for in vivo studies of ACL reconstruction.

Evaluation of an ACL reconstruction takes many forms, one of which is mechanical testing. Mechanical properties of parallel fibered collagenous tissues, such as PT and medial collateral ligament (MCL), are typically determined by uniaxial tensile testing. Due to the characteristic viscoelastic nature of these tissues, the properties may be sensitive to certain variables in the testing procedure, such as strain rate. Although researchers have shown that increasing the strain rate increases the tangent modulus of the ACL [23], few studies have examined the effects on PT. Furthermore, researchers have reported conflicting results in this regard. Danto and Woo, testing rabbit PT at strain rates of 0.016, 1.33, and 135%/s, reported a significantly greater tangent modulus at faster strain rates [15]. Blevins et al., on the other hand, found no significant differences in the tangent modulus of human PT tested at strain rates of 10 or 100%/s [8]. This discrepancy may indicate that the effects of strain rate are species-dependent.

Skeletal maturity is another factor that may affect the mechanical properties of soft tissue determined during uniaxial tensile testing. For example, Haut found an increase in the failure stress of rat tail tendons as donor age increased up to 4 months [16]. Using rabbit MCL, Woo et al. showed a similar trend in tangent modulus [28,29]. Furthermore, skeletal maturity affected the failure mode of rabbit MCL [28,29]. Ligaments from skeletally immature donors failed by avulsion, while those from mature donors failed in midsubstance. Comparable tendencies may exist in PT, although no studies have investigated this possibility.

As the pig knee may be appropriate for in vivo investigations of ACL reconstruction, the current study was designed to further develop the porcine model for this purpose. To characterize changes in the mechanical properties of a graft after implantation in this model, the initial properties of the tissue must be known. Hence, one purpose of this study was to determine baseline mechanical properties of porcine PT. The mechanical properties of soft tissue determined by uniaxial tensile testing may be affected by strain rate and skeletal maturity. Porcine soft tissue, in particular, porcine PT, has not been examined in this manner. Therefore, a second purpose of this study was to investigate the effects of strain rate and skeletal maturity on the mechanical properties of porcine PT.

4.2 Materials and Methods

4.2.1 Specimens

Twenty-eight fresh porcine knees from recently sacrificed animals were used in this study. Seventeen knees were from skeletally immature donors (age: 14-16 weeks), and eleven were from skeletally mature donors as verified by roentgenographic analysis (age: 2-6 years).

4.2.2 Specimen Preparation

The knees were isolated and dissected to leave only the patella-PT-tibia complex intact. The exposed tendon was kept moist throughout preparation and the testing procedure with a 0.9% saline spray. The tibia was potted in a section of PVC pipe with poly(methyl methacrylate). The posterior side of the patella was shaved with a sagittal saw so the bone would fit in a wedge clamp. The medial and lateral portions of the tendon were carefully excised with a scalpel to isolate the central half.

4.2.3 Specimen Fixation

The specimen was loaded in a closed loop hydraulic testing machine (Model 1321, Instron, Canton, MA). Using a custom designed clamp (Figure 4.1), the tibia was fixed to the base of the testing machine at an angle of approximately 30° to the vertical. This angle was selected to assure uniform loading of all fibers in the tendon. The patella was gripped with a wedge clamp attached to the hydraulic actuator arm. The tibia clamp was then positioned with an x-y translator so that the PT was parallel to the direction of axial loading (Figure 4.2).

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Figure 4.1 Custom Designed Clamp

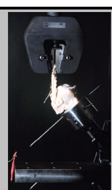

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Figure 4.2 Patellar Tendon Specimen Loaded in Instron

4.2.4 Cross-Sectional Area Determination

A rectangular cross-section was assumed to calculate cross-sectional area of the specimen. After application of a preload of approximately 1 N, width and depth at the mid-point of the specimen were measured three times each with digital calipers. The means of the measurements were used to calculate cross-sectional area.

4.2.5 Mechanical Testing

A differential variable reluctance transducer (DVRT, MicroStrain, Burlington, VT) with a gauge length of 10 mm was inserted at the mid-point of the specimen to measure tissue strain [7] (Figure 4.3). The specimen was conditioned for 20 cycles with a 1.5 mm amplitude triangular wave at an elongation rate of 20 mm/min. The 1 N preload was again applied to the specimen before loading to failure at either 20 mm/min (N=6 immature, 4 mature) or 200 mm/min (N=11 immature, 7 mature). An A/D conversion board (Model Lab-NB, National Instruments, Austin, TX) and LabView 2 software (National Instruments) on a Macintosh LCIII computer were used to record load and DVRT data digitally.

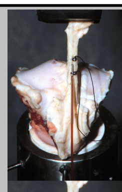

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Figure 4.3 DVRT Inserted in Specimen

4.2.6 Stress Relaxation Analysis

Stress relaxation during specimen conditioning was obtained from the load cell output. The level of cyclic stress relaxation was defined as the ratio of the peak load after equilibrium was reached to the peak load of the first cycle.

4.2.7 Stress-Strain Analysis

The stress-strain curve for each specimen was obtained from the stored digital data. Tissue stress was calculated from the load cell output and the initial cross-sectional area of the specimen. Tissue strain was calculated from the DVRT output and the initial transducer length. Tangent modulus, ultimate stress and strain, and failure mode were noted. Tangent modulus was defined as the slope of the linear portion of the stress-strain curve between 2 and 4% strain. Ultimate stress was defined as the maximum value of stress attained during testing, and ultimate strain was defined as the strain at this point. All values are reported as mean ± standard error of the mean.

4.3 Results

The cross-sectional areas determined for immature and mature specimens were 8.8 ± 0.4 and 11.9 ± 0.8 mm^2, respectively. Calculated strain rates corresponding to elongation rates of 20 and 200 mm/min were 0.22 ± 0.02 and 2.17 ± 0.14%/s, respectively. A representative stress versus time curve obtained during specimen conditioning is shown in Figure 4.4. This curve displays the typical relaxation behavior of a viscoelastic material when it is cycled over a fixed deformation. The level of cyclic stress relaxation during conditioning was significantly different for immature and mature specimens (81 ± 1.5 and 87 ± 1.5% , respectively) ($p<.05$). Representative stress-strain curves obtained during failure testing are shown in Figure 4.5. These curves display the nonlinear toe region, linear region, and nonlinear region prior to failure typically seen in the stress-strain relationship of these viscoelastic soft tissues.

Skeletal maturity significantly affected the failure mode of the tendons. Immature specimens failed either in midsubstance or by bony avulsion, while all mature specimens failed in midsubstance. Strain rate significantly affected the failure mode of only immature specimens. At the slower rate, all immature specimens failed by bony avulsion from either the patella or tibia. At the faster rate, 7 of 11 immature specimens failed in the midsubstance. The other immature specimens elongated at this rate failed by bony avulsion from the tibia.

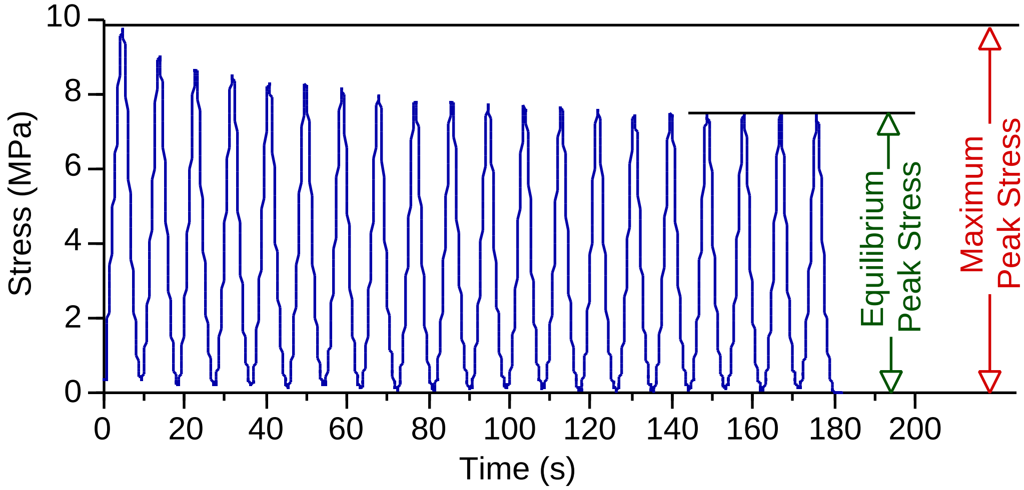

Figure 4.4 Representative Conditioning Stress-Time Plot

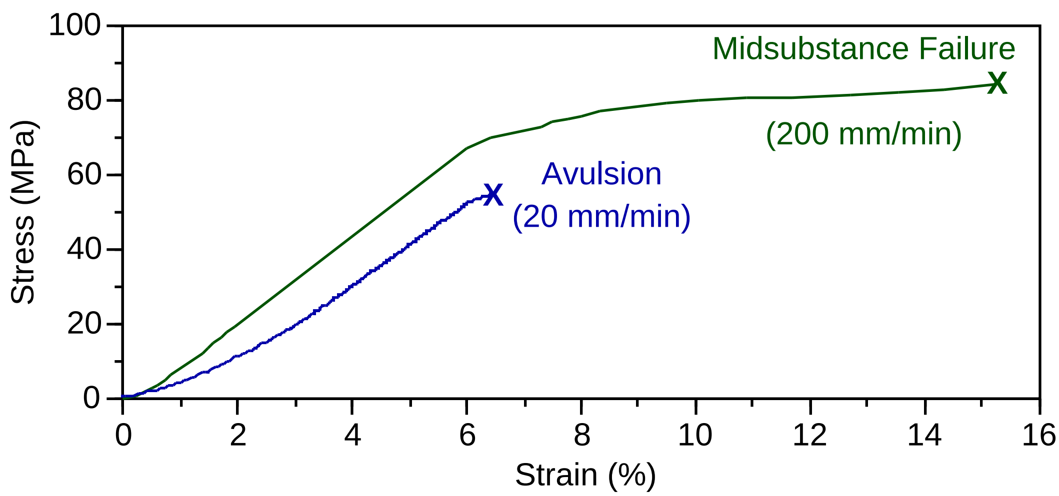

Figure 4.5 Representative Failure Stress-Strain Curves

Ultimate properties can be reported only for specimens that failed in the midsubstance. Therefore, ultimate properties were compared using two t-tests: one between immature and mature specimens elongated at the faster rate, and one between the different elongation rates for mature specimens. Ultimate stress was significantly greater for mature specimens when compared with immature specimens ($p<.01$) (Figure 4.6). Ultimate stress for mature specimens was also significantly greater at the faster elongation rate than at the slower rate ($p<.01$). Ultimate strain was not significantly affected by strain rate ($p=.23$) nor maturity ($p=.82$).

Because tangent modulus was determined for all specimens prior to failure, a two-way ANOVA was used to make statistical comparisons between all groups. Both strain rate and skeletal maturity significantly affected the tangent modulus ($p<.01$) (Figure 4.7).

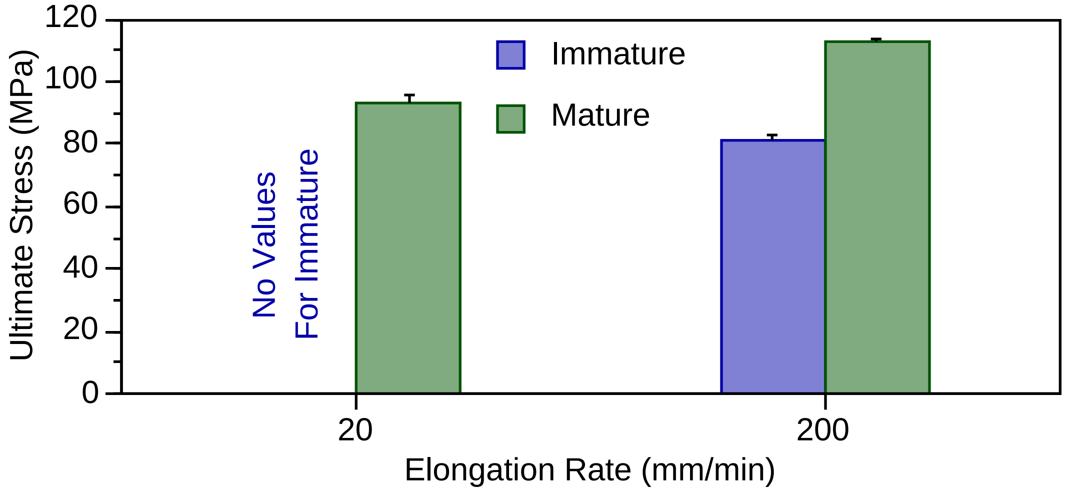

Figure 4.6 Effects of Strain Rate and Skeletal Maturity on Ultimate Stress

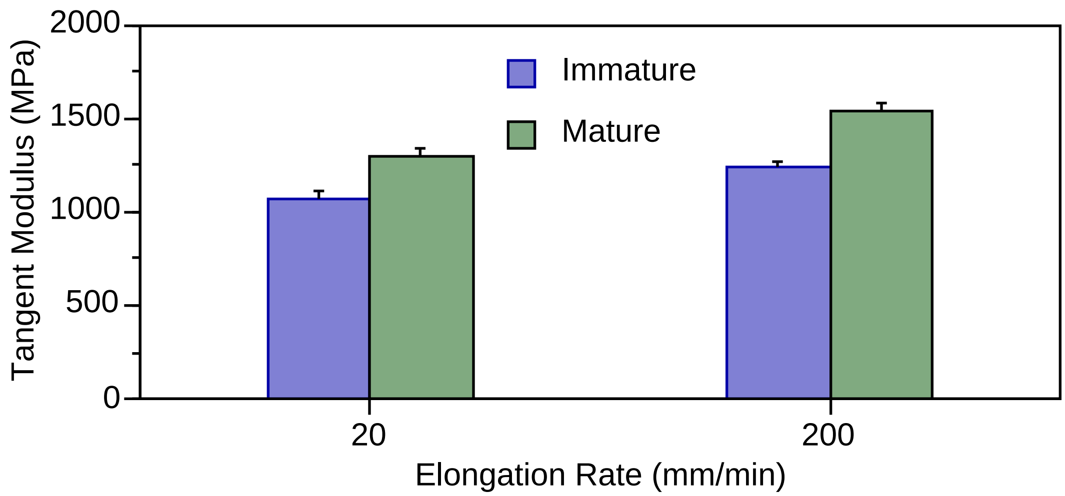

Figure 4.7 Effects of Strain Rate and Skeletal Maturity on Tangent Modulus

4.4 Discussion

This study examined the effects of strain rate on the mechanical properties of skeletally immature and mature porcine PT determined during uniaxial tensile testing. For the experiment, one elongation rate was chosen to provide a relatively low strain rate, while a second, faster elongation rate was selected to provide a moderate strain rate. Mechanical properties of the PT were evaluated by using an implantable transducer to measure tissue strain, measuring the dimensions of an assumed cross-section to calculate specimen area, and measuring the load applied across the specimen. Different levels of stress relaxation between immature and mature specimens was attributed to a decrease in water content with skeletal maturity. Significant differences in the tangent modulus, ultimate stress, and failure mode of the tendons were attributed to a change in strain rate and to the level of skeletal maturity.

The level of cyclic stress relaxation during conditioning was significantly different between immature and mature specimens. Immature specimens relaxed to a greater degree than mature specimens did. This may be explained by a difference in water content between immature and mature tendons. Chimich et al. reported greater cyclic relaxation in rabbit medial collateral ligaments with higher water content than in those with lower water content [12]. Immature ligaments have been reported to have a higher water content than mature ligaments [4]. Therefore, it is expected that immature tendons will exhibit greater cyclic stress relaxation, as was noted in the current study.

Mechanical properties of PT from various species that have been reported in the literature are summarized in Table 4.1. Using grip-to-grip displacement to calculate tissue strain may lead to a misleading tangent modulus and ultimate strain [22,27,32,34]. Therefore, these properties are included only for studies which used methods to determine local tissue strain. Because ultimate stress is not affected by this measurement technique, values are included from all reviewed studies. The tangent modulus, ultimate stress, and ultimate strain of porcine PT determined in the current study fall in the mid to upper range of values reported for other species. In particular, the tangent modulus and ultimate stress of porcine PT are approximately twice those of human PT as determined by Johnson et al. [20].

Researchers have attributed an avulsion failure of a ligament during uniaxial testing to either a low strain rate or to skeletal immaturity [30]. Skeletal maturity appears to be the more dominant of the two factors. For example, using rabbit MCL, Woo et al. found that skeletal maturity affected failure mode, but strain rate did not [29]. The authors theorized that proximity to the open epiphysis made the tibial insertion site the weakest link in the bone-ligament-bone complex of immature donors, and the specimens failed by avulsion. When skeletal maturity was reached, the strength of the insertion site surpassed that of the ligament, and midsubstance failure became the typical failure mode. The current study similarly found skeletal maturity to greatly affect the failure mode of porcine PT. It is important to note, however, that midsubstance failure occurred in most immature specimens when strain rate was increased. This finding is contrary to immature rabbit MCL, which always failed by avulsion regardless of strain rate. Therefore, although skeletal maturity may be the dominant factor, strain rate may yet play a significant role in the failure mode of some soft tissues, particularly those from immature donors.

Table 4.1 Reported Mechanical Properties of Patellar Tendon From Various Species

Species	#	Elongation Rate (mm/min)	Strain Rate (%/s)	Tangent Modulus (MPa)	Ultimate Stress (MPa)	Ultimate Strain (%)
Human (younger) [20]	15	200	--	660 ± 69	64.7 ± 3.9	14 ± 2
Human (older) [20]	15	200	--	504 ± 57	53.6 ± 2.6	15 ± 1
Human* [14]	5	3000	--	--	97.5 ± 3.9	--
	5	3000	--	--	95.5 ± 7.5	--
	5	3000	--	--	77.6 ± 7.9	--
	4	3000	--	--	92.7 ± 10.0	--
Human [8]	41	--	10†	--	35.9 ± 1.7	--
	40	--	100†	--	37.1 ± 1.9	--
Human [22]	7‡	--	100	306 ± 59	58.3 ± 6.1	12.0 ± 2.6
Human [11]	3	--	100	--	68.5 ± 6.0	--
Primate [9]	5	--	100	443 ± 43	86.0 ± 12.4	29.6 ± 3.6
Rabbit [32]	14	20	--	1390 ± 53	57.1 ± 2.5	5.3 ± 0.2
Rabbit [15]	6	0.48	0.016 ± 0.001	955 ± 97	--	--
	6	49.8	1.33 ± 0.15	1637 ± 132	--	--
	6	6780	135 ± 12	1855 ± 77	--	--
Porcine (immature)	6	20	0.23 ± 0.02	1075 ± 40	--	--
	11§	200	1.92 ± 0.10	1246 ± 22	81.4 ± 1.1	13.9 ± 1.8
Porcine (mature)	4	20	0.21 ± 0.02	1292 ± 46	93.1 ± 2.2	16.7 ± 2.1
	7	200	2.57 ± 0.26	1537 ± 38	112.4 ± 1.5	13.4 ± 1.3

Note: Values are mean ± standard error of the mean.

*PT grafts of different widths were tested. †Strain rate was calculated from grip-to-grip displacement. ‡Ultimate strain was reported for 4 specimens. §Ultimate properties calculated only for specimens that failed in midsubstance (N=7).

The failure mode of mature PT may be species-dependent. Danto and Woo reported avulsion failures from the patella in 94% (17 of 18) of mature rabbit PT specimens tested [15]. Blevins et al. reported tibial avulsions in 12% (10 of 82) and patellar avulsions in 2% (2 of 82) of human PT specimens [8]. Forty-six percent (38 of 82) of the specimens failed in midsubstance. The current study consistently found midsubstance failure in mature specimens. In none of these studies was the failure mode of mature specimens affected by strain rate. Because specimens were kept moist by a saline spray in all three studies, the differences cannot be attributed to testing environment. Instead, perhaps anatomical differences, such as patella size and shape, lead to different fibrocartilage structures in the insertion sites. Hence, the relative strengths of the insertion sites in some species may be greater than those in other species.

Strain rate was found to significantly affect the tangent modulus of porcine PT. A ten-fold increase in strain rate increased the tangent modulus of immature porcine PT by 16%, and that of mature specimens by 19%. This finding is similar to those reported for the tangent modulus of skeletally immature rabbit MCL [29] and of skeletally mature rabbit PT [15] and MCL [29]. However, the tangent modulus of human PT was not affected by strain rate [8]. Therefore, the possibility remains that the effects of strain rate on the tangent modulus and ultimate stress of PT are species-dependent. Another possibility for these different findings is the level of tissue hydration. Depending on the level of tissue hydration, strain rate may or may not affect the mechanical properties of PT [17].

Strain rate also significantly affected the ultimate stress of skeletally mature porcine PT. A ten-fold increase in strain rate increased the ultimate stress by 21%. This response has been noted for rat tail tendons [16]. However, the ultimate stress of human PT [8] and mature rabbit MCL [29] was not significantly affected by strain rate. These varying results indicate that effects of strain rate on the ultimate stress may be both tissue-dependent and species-dependent. Again, differences in tissue hydration between the studies may also offer an explanation.

The mechanical properties of skeletally immature and mature porcine PT were determined for two strain rates. Tangent modulus, ultimate stress, and failure mode were significantly affected by strain rate and by skeletal maturity. Therefore, both strain rate and skeletal maturity are important considerations in the tensile testing of porcine PT. Future studies using porcine PT will examine the effects of other variables in the testing procedure on the mechanical properties to provide a greater understanding of this tissue for use in an in vivo ACL reconstruction model.