

**Shear Wave Tensiometry Tracks Reductions in Collateral Ligament Tension Due to
Incremental Releases**

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Abstract

Surgeons routinely perform incremental releases on overly tight ligaments during total knee arthroplasty (TKA) to reduce ligament tension and achieve their desired implant alignment. However, current methods to assess whether the surgeon achieved their desired reduction in the tension of a released ligament are subjective and/or do not provide a quantitative metric of tension in an individual ligament. Accordingly, the purpose of this study was to determine whether shear wave tensiometry, a novel method to assess tension in individual ligaments based on the speed of shear wave propagation, can detect changes in ligament tension following incremental releases. In seven medial and eight lateral collateral porcine ligaments (MCL and LCL, respectively), we measured shear wave speeds and ligament tension before and after incremental releases consisting of punctures with an 18-gauge needle. We found that shear wave speed squared decreased linearly with decreasing tension in both the MCL ($r^2_{\text{avg}} = 0.76$) and LCL ($r^2_{\text{avg}} = 0.94$). We determined that errors in predicting tension following incremental releases were 24.5 N and 12.2 N in the MCL and LCL, respectively, using specimen-specific calibrations. These results suggest shear wave tensiometry is a promising method to objectively measure the tension reduction in released structures. **Clinical Significance:** Direct, objective measurements of the tension changes in individual ligaments following release could enhance surgical precision during soft tissue balancing in TKA. Thus, shear wave tensiometry could help surgeons reduce the risk of poor outcomes associated with overly tight ligaments, including residual knee pain and stiffness.

Keywords

Total knee arthroplasty, ligament tensiometry, soft tissue balancing, intraoperative sensor, ligament release

Introduction

During total knee arthroplasty (TKA), surgeons routinely have to address overly tight ligaments. The traditional alignment targets in TKA (i.e., mechanical alignment with equal and rectangular gaps) often cause the surgeon to alter the patient's native alignment and gaps, creating overly tight ligaments.¹⁻⁴ Additionally, prior studies suggest that ligaments in the diseased compartment (e.g., the medial collateral ligament in a knee with osteoarthritis in the medial compartment) contract and/or become stiffer.^{5,6} Thus, to achieve their desired implant alignment and soft tissue balance,^{2,7} surgeons commonly reduce ligament tension using a

ligament release technique called “pie crusting”. Pie crusting, or the “inside-out” technique, involves iteratively puncturing the overly tight ligament with a needle or scalpel to reduce the tension in that ligament.^{8,9} Previous *ex vivo*¹⁰⁻¹⁴ and *in vivo*^{8,15} studies have shown that pie crusting does reduce the tension in ligaments, but tracking these reductions in tension remain challenging. Indeed, improper ligament tensioning is one of the primary factors associated with knee stiffness and pain,^{16,17} which are two common reasons patients report not being satisfied following TKA.¹⁸ With up to 19% of patients reporting that they are not satisfied following TKA,^{16,17,19,20} a method to track ligament tension reductions during iterative release procedures is needed to mitigate the risk of improper ligament tension post-operatively.

Current methods to assess reductions in ligament tension during these releases are subjective and/or not capable of directly measuring the tension in an individual ligament. Laxity assessments,^{21,22} gap tensioners,^{23,24} and spacer blocks²⁵ are most commonly used to assess ligament tension during TKA. However, many ligaments work in parallel to determine joint laxity, and clinical assessments of laxity are often qualitative. A recent study showed that assessing ligament tension based on joint gaps alone, one common measure of joint laxity, resulted in average tibiofemoral contact forces up to 350 N,²⁶ which are about three-times greater than those in the native knee.²⁷ An alternative to laxity and gap assessments are instrumented trials that measure the tibiofemoral contact forces;^{18,28} however, these trials provide quantitative guidance about the tensions of all the ligaments in a compartment rather than the tension in a particular ligament. Thus, a direct and objective method to track changes in tension in the released ligament would enhance the surgeon’s ability to determine when they have achieved a desired reduction in tension during iterative releases.

One promising method to measure the tension in individual ligaments during TKA directly and objectively is shear wave tensiometry.^{29,30} Prior studies have shown that shear wave speed squared is proportional to the axial stress in tendons²⁹ and ligaments.³⁰ This relationship between shear wave speed squared and axial stress is consistent with the analytical relationship predicted by a tensioned beam model.²⁹ However, it is unknown whether reductions in tension due to releases alter shear wave propagation speed differently than reductions in tension due to changes in the axial stretch of an intact ligament. Thus, a pre-requisite step to translating shear wave tensiometry into the operating room to monitor ligament tension during soft tissue balancing is to determine whether shear wave speeds track ligament tension changes following iterative releases.

Accordingly, we had two objectives for this study. Our first objective was to characterize the relationship between shear wave speed and tension in medial and lateral collateral ligaments (MCL and LCL, respectively) following incremental releases. We chose to study the MCL and LCL because collateral ligament tension is a common focus of soft tissue balancing during TKA. Our second objective was to determine the errors in predicting tension using shear wave speeds during incremental releases in the MCL and LCL.

METHODS

Specimen preparation

We procured eight MCLs and eight LCLs from a crossbreed of large white, landrace, and red duroc pigs for this study (weight = 139.0 ± 6.8 kg, age = 6 months). One MCL failed during testing and was excluded. We used porcine collateral ligaments for this study because MCLs

from pigs of this size have been previously shown to have similar structural (e.g., stiffness, failure deformation) and material (e.g., modulus, failure strain) properties to those of human MCLs.³¹

Specimen preparation consisted of the following steps. We first exposed each ligament by removing all superficial tissue, and then flexed each knee (i.e., stifle joint) to the angle where the fibers across the ligament appeared to be most uniformly taught; this angle was typically around 80° of flexion.³¹ Next, we cut bone blocks containing the ligament attachments from the distal femoral condyle and proximal tibia/fibula using an oscillating saw. We then secured each bone block in an aluminum cup using a potting material (Bondo Fiberglass Resin, 3M, St. Paul, MN). Finally, we dissected away excess tissue superficial and deep to the outer surface of the ligament prior to experimentation.

Experimental procedure

Following dissection, we mounted each specimen for experimentation using the following steps. First, we secured the potting cups in the grips of an electrodynamic testing system (Acumen 3, MTS, Eden Prairie, MN) with the specimen aligned visually with the loading axis. We fine-tuned the orientation of the potting cups to achieve the most-uniform tension across the width of the ligament. Next, we mounted the three components of the shear wave tensiometer, which housed the piezoelectric tapping device and two accelerometers, in a 3D-printed structure that pressed each component against the outer surface of the ligament with a controlled force of 1.4 ± 0.2 N (Figure 1). The level of this controlled force was set to minimally influence the shear wave speeds.³² We measured the controlled force of each component using a load cell (LC101, Omegadyne Inc., with reported linearity = 0.25 N). The piezoelectric tapping device (PK4JQP1, Thorlabs Inc, Newton, NJ) induced shear waves in each specimen by

delivering 20- μ m impulsive taps across the width of the ligament at a tap rate of 10 Hz. The pair of single-axis accelerometers (Model 352C23, PCB Piezotronics), spaced 6.5 mm apart, tracked the shear wave propagation.²⁹ Further details on the tapping device and the accelerometer arms are described in the Supplemental materials (Supplement A, Figures S-1, S-2, and S-3). We completed two repeatability studies using this apparatus to mimic (1) setting the apparatus up on different days and (2) reapplying contact of the tapper and accelerometer arms on the ligament between experimental trials (Supplement B, Figures S-4 and S-5). We determined the repeatability limits³³ for the first scenario to be 6.70 m/s for the MCL and 4.58 m/s for the LCL, and those for the second scenario to be 1.49 m/s for the MCL and 2.70 m/s for the LCL.

Before testing, we preconditioned each ligament with the following procedure. First, we axially loaded each ligament with 50 cycles from 25 to 250 N at 1 Hz followed by a static hold at 250 N for 30 minutes to minimize viscoelastic effects.³⁰ We limited the maximum load to 250 N based on pilot studies that showed failures at the bony attachments of some porcine ligaments when applied loads exceeded 250 N. We recorded the maximum stretch during the 30-minute static hold at 250 N, referred to subsequently as δ_{250} .

Following preconditioning, we measured shear wave speeds before and after sets of incremental releases. First, we cycled each ligament 10 times between its slack length and δ_{250} at 1 Hz, and then we measured the shear wave speeds with the intact ligament held to a stretch of δ_{250} . We then repeated three steps (i.e., release, cycle, and measure) to characterize changes in shear wave speed following incremental releases (Figure 2). In the release step, we stretched the ligament to δ_{250} and performed a set of incremental releases. Each set of releases consisted of a minimum of five punctures across the ligament width using an 18-gauge needle. We performed more than five punctures for some trials to ensure that the tension in the ligament stretch to δ_{250}

dropped by at least 10 N in each set of releases. We performed subsequent release sets about 2 mm above the previous row of punctures. In the cycle step, we cycled the ligament 10 times between its slack length and δ_{250} at 1 Hz to simulate the surgeon intraoperatively flexing and extending the knee following release (i.e., pie crusting). In the measure step, we held the ligament at a stretch of δ_{250} and placed the tapper and accelerometer arms on the surface of the ligament as described previously. We held the ligament at a stretch of δ_{250} for 10 seconds while measuring both the shear wave speed and applied axial tension. We repeated this release-cycle-measure procedure until the tension in the ligament when stretched to δ_{250} was below 50 N. Throughout testing, we kept the ligaments moist by regularly spraying them with 0.9% saline.

Post-processing

We post-processed the recorded accelerometer signals to obtain the data used in later analyses. We computed the shear wave speeds following each tap by measuring the time delay between the arrivals of the shear waves at the two accelerometers. We filtered waveforms using a second-order Butterworth bandpass filter (MATLAB R2020b) with lower and upper cutoff frequencies of 150 and 2500 Hz, respectively. To determine the time delay in shear wave arrival between accelerometers, we computed the normalized cross-correlation between the first accelerometer waveform (i.e., the template signal was the first 0.8 ms of the transient response after tap onset) with the second accelerometer waveform (i.e., a search region that extended to 1.0 ms after tap onset).²⁹ The time shift that maximized the normalized cross-correlation was determined. We performed a sub-sample interpolation using a local 3-point cosine fit of the normalized cross-correlation values.³⁴ We computed the shear wave speeds by dividing the spacing between the accelerometers (6.5 mm) by the time delay in wave arrival. The tension was

ascertained by averaging the data from the MTS load cell (661.18E-02, MTS, reported hysteresis/non-linearity = 0.08% full scale) across the 10-second measurement.

Statistical analysis

To characterize the relationship between shear wave speed and tension, we performed simple linear regressions for each ligament type with shear wave speed squared as the independent variable and tension as the dependent variable. Although the analytic relationship is between shear wave speed squared and axial stress, we chose to use tension in the present study because (1) tension is a more understandable quantity for clinicians, and (2) our ligaments all had similar cross-sectional areas so using tension instead of axial stress only scaled the slope of our fits. The level of significance, α , was set to 0.05. With at least seven ligaments of each type, strong relationships between shear wave speed squared and tension (i.e., $r^2 = 0.64$) could be detected with $\alpha = 0.05$ and power, $1-\beta$, set to 0.8.³⁵

To determine the errors in measuring tension after releases, we evaluated three different cases. The first case was predicting the tension in a ligament using shear wave speed data from both MCLs and LCLs. For this first case, we performed a leave-one-out analysis in which we performed a linear regression between shear wave speed squared and tension from 14 ligaments and then used this regression equation to predict the tension in the 15th ligament. We repeated this procedure for all combinations of 15 ligaments, and characterized the resulting errors in tension by the mean (bias), standard deviation (precision), and root-mean-square error (RMSE) (ISO 5725-1, ASTM E177-13).^{33,36} The second case was predicting tension in a ligament using data from the same ligament type (e.g., LCL tension predicted only from LCL data). For this second case, we also performed a leave-one-out analysis as described above, but we performed the linear regression using data from all but one MCL or LCL and then predicted tension in the

MCL or LCL not included in the linear regression, respectively. The third case was predicting tension using data from that same ligament. For this third case, we determined the bias, precision, and RMSE of the residual errors from the linear regression of each ligament.

RESULTS

The shear wave speed squared of both the MCL and the LCL decreased linearly with decreasing tension (Figure 3). The mean $\pm$ standard deviation of the coefficients of determination (r^2) of the individual MCLs and LCLs were 0.76 ± 0.18 and 0.94 ± 0.07 , respectively (individual data shown in Supplement C, Figures S-6 and S-7). Within each ligament type, the r^2 values for fits to the pooled data of each ligament decreased to 0.58 and 0.73 for the MCL and LCL, respectively.

The error analysis showed that the errors in the predicted tension using shear wave speed were smallest when using ligament-specific data compared to using data from other ligaments (Tables 1 and 2). For the MCL, the average RMSEs of predicted tension were 40.3 N (16.1% of 250-N max) when predicting based on data from all other MCLs and LCLs, 40.9 N (16.4% of 250-N max) when predicting based on data from all other MCLs, and 24.5 N (9.8% of 250-N max) when predicting based on data from the specific MCL (Table 1). For the LCL, the average RMSEs of predicted tension were 41.5 N (16.6% of 250-N max) when predicting based on the data from all other MCLs and LCLs, 43.4 N (17.4% of 250-N max) when predicting based on data from all other LCLs, and 12.2 N (4.9% of 250-N max) when predicting based on data from the specific LCL (Table 2).

DISCUSSION

The objectives of the study were to (1) characterize the relationship between shear wave speed squared and tension in porcine collateral ligaments following incremental releases and (2) determine the errors in predicting tension from shear wave speeds during incremental releases of porcine collateral ligaments. The first key finding was that there were strong, linear relationships between shear wave speed squared and tension in both the MCL and the LCL following incremental releases. The second key finding was that ligament tension could be predicted from the measured shear wave speeds to within 24.5 N for the MCL and 12.2 N for the LCL.

Regarding the first key finding, we demonstrated that the tensioned beam model,²⁹ shown previously to hold in intact ligaments,³⁰ also applies to ligaments damaged by iterative releases. Our extension of shear wave tensiometry to ligaments damaged by needle punctures suggests that our technique could also work in ligaments damaged by other means (e.g., ligament sprain). The individual r^2 -values for the LCL (0.94 ± 0.07) are consistent with previous studies using shear wave tensiometry to measure intact MCLs and LCLs ($r^2 = 0.94$ and 0.98 , respectively) and porcine digital flexor tendons (mean $r^2 = 0.96$ - 0.98).^{29,30,37} However, while the individual r^2 -values for the MCL are still moderately strong (0.76 ± 0.18), the values are lower than the LCL and previous studies on intact ligaments. One possible explanation for these lower r^2 -values in the MCL compared to those in the LCL is that the fibers in the MCL might engage more non-uniformly than fibers in the LCL. We are not aware of any studies that have characterized ligament fiber engagement in porcine collateral ligaments, but assuming that the engagement patterns are similar to those in human collateral ligaments, the fibers of the MCL will engage less uniformly across the width of ligament compared to those in the LCL.³⁸ This non-uniform engagement of fibers in the MCL could increase the variability of the measured shear wave

speeds after releases because each puncture could have a different impact on shear wave propagation depending on whether the needle severed a tighter or looser fiber. Investigating these regional differences is beyond the scope of the present study, but regional differences are a topic of ongoing research in our lab.

The range of shear wave speeds in the present study were lower than that in a previous study with intact porcine ligaments.³⁰ In the present study, the shear wave speeds ranged from 14.3 to 46.5 m/s, whereas Blank et al. measured shear wave speeds in intact ligaments to be 32.5 to 160.3 m/s for the same load range.³⁰ The primary difference between the two studies is the sensors used to track shear wave propagation. Blank et al. tracked shear wave propagation with laser Doppler vibrometers,³⁰ a non-contact measurement technique. We tracked shear wave propagation with accelerometers, a contact measurement technique. The contacting accelerometers likely increase inertia and damping present in the system. The tensioned beam model suggests that the effective material density is the constant of proportionality between shear wave speed squared and stress.²⁹ Effective material density includes both the material density and inertia of the surroundings. For example, a previous study showed that shear wave speeds in porcine digital flexor tendons immersed in a saline were 22% less than those measured in air.³⁷ In the present study, we expect the added mass of the tensiometer components (Figure 1) to be greater than that of saline surrounding the tissue, which likely explains why the range of our shear wave speeds are on average 75% lower than those measured with laser Doppler vibrometers in air.³⁰ Inherent damping due to the contact tensiometer may also alter the frequency content of the vibration measured at the two sensors, and thereby alter the wave speed as ascertained from the transient vibrations. The potential added-mass and damping effect indicates that the shear wave speeds measured are dependent on the tensiometer design, and care

must be taken to consider such effects when interpreting wave speed measures in tensioned tissues.

Regarding the second key finding, our errors in predicting tension in a released ligament (12.2 to 43.4 N) are comparable to the current gold standard intraoperative measurements of tibiofemoral contact force (VERASENSE, OrthoSensor, Inc).³⁹ A recent study showed that the best-case RMSE for contact force of the femur on the tibia within a compartment measured by the VERASENSE was 13.8 N.⁴⁰ During passive flexion, the tensions in the ligaments largely determine the contact forces of femur on the tibia. Thus, with comparable errors in predicting load and the advantage of being able to measuring the tension in individual ligaments, shear wave tensiometry is a promising approach to intraoperative monitoring of ligament tension during release.

Three limitations should be considered when interpreting our findings. First, we performed these measurements in porcine ligaments rather than human ligaments. The human MCL, in particular, has a greater aspect ratio (average human = 15.3⁴¹ and average porcine MCL = 3.0; Supplement D, Tables S-1 and S-2), which may lead to a greater non-uniformity of fiber engagement. Further investigation is needed to ascertain whether the larger aspect ratio results in more variable effects of a needle puncture. The similar mechanical behavior between human and porcine MCLs³¹ suggests that human ligaments would also demonstrate a predictable reduction in shear wave speed with ligament releases, but that the absolute changes in shear wave speed may differ from those determined in the present study. Second, we studied isolated ligaments in the present study. Thus, the effects of surrounding structures were not included in this initial study because we wanted to focus on the changes in the ligament without potential confounding effects. As described above,^{29,37} the surrounding tissues/implants *in vivo* might further decrease

the range of shear wave speeds. Now that we have shown that the relationship between shear wave speed and tension holds following release, we can explore whether this relationship changes *in situ* in future studies. Third, achieving the minimum errors reported requires a ligament-specific calibration. Because shear wave tensiometry has been shown to track tension using shear wave speed squared in both intact³⁰ and released ligaments, one possible option to calibrate shear wave speeds in a particular ligament that is to be released is to calibrate the shear wave speed-tension relationship during a laxity assessment before release. The tension in the ligament pre-release could be estimated based on the applied load and the patient's anatomy.⁴² The shear wave speed-tension relationship pre-release could then be determined using a regression between squared shear wave speed and the estimated tension, and this pre-release relationship could then be used to determine reductions in tension following iterative releases.

In conclusion, this study showed that shear wave tensiometry can track reductions in tension due incremental releases of the MCL and LCL within 24.5 N and 12.2 N, respectively (9.8 to 4.9% of the initial 250-N tension). The strength of the relationship between shear wave speed and tension was similar following release to that seen previously in intact ligaments.³⁰ Thus, using shear wave tensiometry to measure tension is a promising approach to guide incremental ligament releases to achieve a desired reduction in an overly tight ligament.

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Table 1. Results from our error analysis of predicted MCL tension using data from all other LCLs and MCLs, using data from all other MCLs, and using data from the specific MCL. The values shown are the mean (bias), standard deviation (precision), and root-mean-square error (RMSE).

Predicted Specimen	Error in Predicted Tension (N)								
	MCLs and LCLs			MCLs			Specific MCL		
	Bias	Precision	RMSE	Bias	Precision	RMSE	Bias	Precision	RMSE
MCL 1	29.2	37.0	36.2	31.9	39.2	38.6	21.4	27.4	26.5
MCL 2	16.5	21.4	21.4	20.3	25.5	29.2	12.3	16.8	15.9
MCL 3	40.8	41.0	49.6	39.9	42.0	43.7	36.6	40.7	38.7
MCL 4	27.6	39.3	38.4	28.3	40.6	42.9	27.1	38.8	37.4
MCL 5	17.1	22.7	21.4	19.3	25.8	25.3	15.0	21.9	20.9
MCL 6	70.5	32.9	77.2	56.8	25.0	61.6	11.7	14.7	14.0
MCL 7	29.2	34.1	38.2	33.1	36.8	44.6	16.6	19.2	18.1
Average	33.0	32.6	40.3	32.8	33.6	40.9	20.1	25.6	24.5

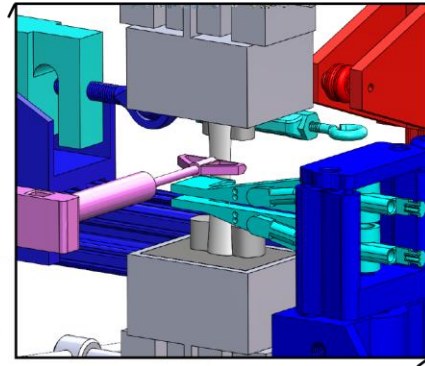
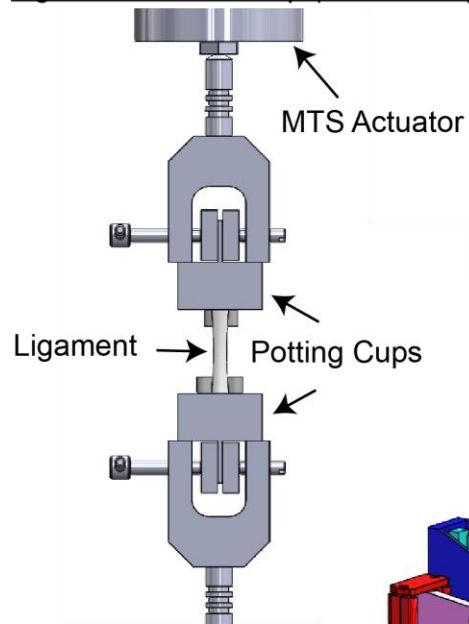
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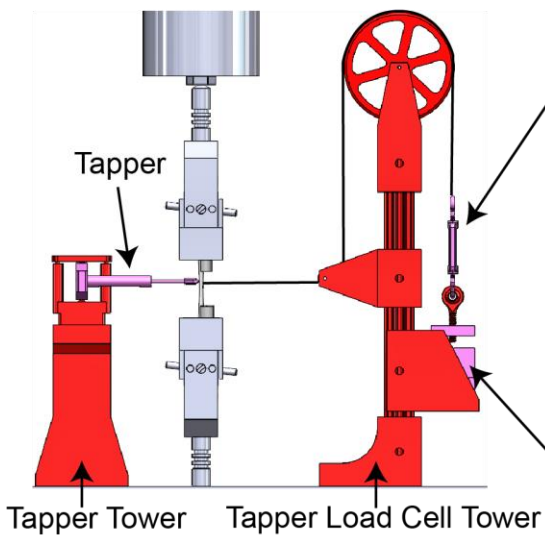
Table 2. Results from our error analysis of predicted LCL tension using data from all other LCLs and MCLs, data from all other LCLs, and data from the specific LCL. The values shown are the mean (bias), standard deviation (precision), and root-mean-square error (RMSE).

Predicted Specimen	Error in Predicted Tension (N)								
	MCLs and LCLs			LCLs			Specific LCL		
	Bias	Precision	RMSE	Bias	Precision	RMSE	Bias	Precision	RMSE
LCL 1	17.8	25.7	25.0	43.8	24.6	49.8	15.2	21.4	20.8
LCL 2	35.4	41.3	43.3	28.9	30.7	33.7	12.7	15.9	14.9
LCL 3	26.5	33.9	32.5	16.1	21.3	21.2	8.7	11.0	10.2
LCL 4	42.4	40.3	52.6	33.9	30.0	43.1	5.8	9.6	9.4
LCL 5	21.0	26.3	25.2	23.7	10.7	25.8	7.0	9.8	9.4
LCL 6	52.3	24.9	57.0	81.0	64.1	99.9	3.9	4.8	4.3
LCL 7	35.4	27.9	42.6	24.8	13.2	27.8	7.8	9.8	9.3
LCL 8	41.4	52.7	54.1	35.7	45.1	46.0	16.6	20.5	19.4
Average	34.0	34.1	41.5	36.0	29.9	43.4	9.7	12.8	12.2

Ligament in MTS Grip (Front View)



Tapper Tensioning Fixture (Side View)



Accelerometer Tensioning Fixture (Front View)

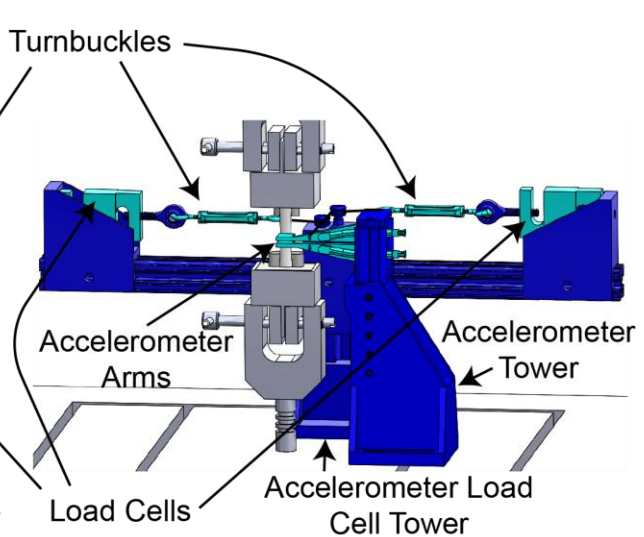


Figure 1. Renderings of CAD assembly show the test apparatus used to control the application forces of the tensiometer components against the ligament (middle). The bony attachments of each ligament were fixed within two potting cups mounted to the MTS (top left). The tensiometer components included two 3D-printed arms holding accelerometers and a tapper (top right). The Tapper Tensioning Fixture (bottom left) included a tapper mounted to the tapper tower and a load cell attached to the tapper load cell tower. The tapper and load cell were connected by a turnbuckle and fishing line (Dorisea Extreme Braid 0.63 mm, not pictured in the middle image for clarity), and the turnbuckle was used to adjust the tension in the fishing line to control the application force of the tapper against the ligament. The tapper, load cell, and turnbuckle are shown in pink, and the tapper tower and tapper load cell tower are shown in red. The Accelerometer Tensioning Fixture (bottom right) included two accelerometer arms mounted to the accelerometer tower and two load cells attached to the accelerometer load cell tower. Each accelerometer arm was connected to its own load cell with a turnbuckle and fishing line. Like in the Tapper Tensioning Fixture, a turnbuckle was used to adjust the tension in each fishing line to control the application force of each accelerometer arm against the ligament. The accelerometer arms, load cells, and turnbuckles are shown in cyan, and the accelerometer tower and accelerometer load cell tower are shown in blue.

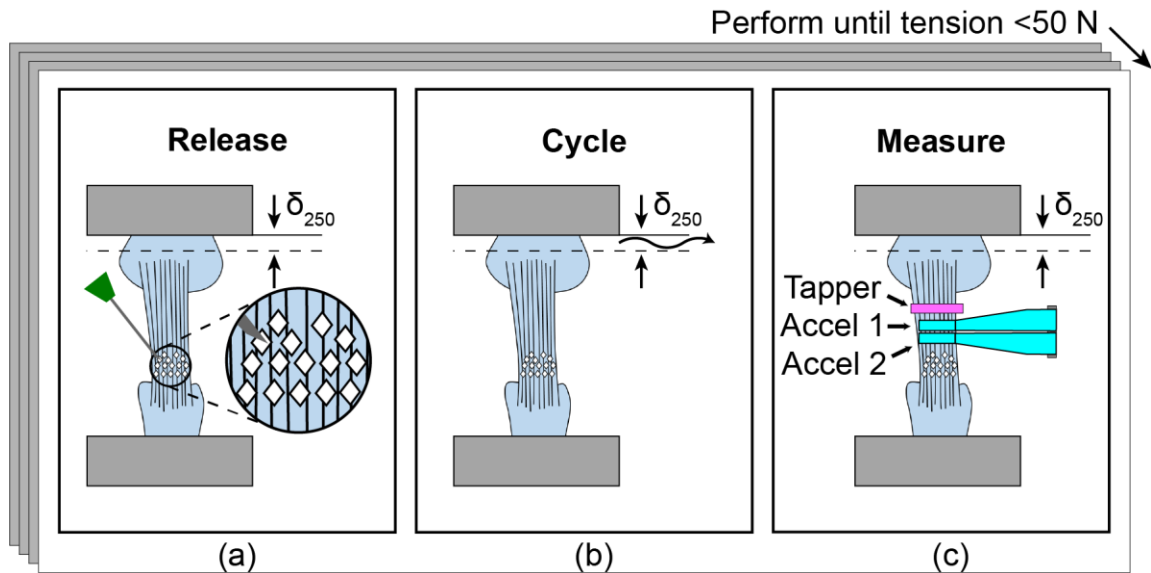


Figure 2. The experimental procedure to characterize changes in shear wave speed following incremental releases included three steps: **(a) Release:** the ligament was ramped to the maximum displacement under 250 N tension, δ_{250} , which we measured during preconditioning. The ligament was punctured at least 5 times with an 18-gauge needle until tension in the ligament decreased by at least 10 N. **(b) Cycle:** the ligament was cycled between slack length and δ_{250} for 10 cycles at 1 Hz. **(c) Measure:** the shear wave tensiometer was used to measure the shear wave speed during a 10-second static hold using the tapper to excite a shear wave and arms with embedded accelerometers to measure the shear wave propagation. This process was repeated until the tension in the ligament at δ_{250} dropped below 50 N.

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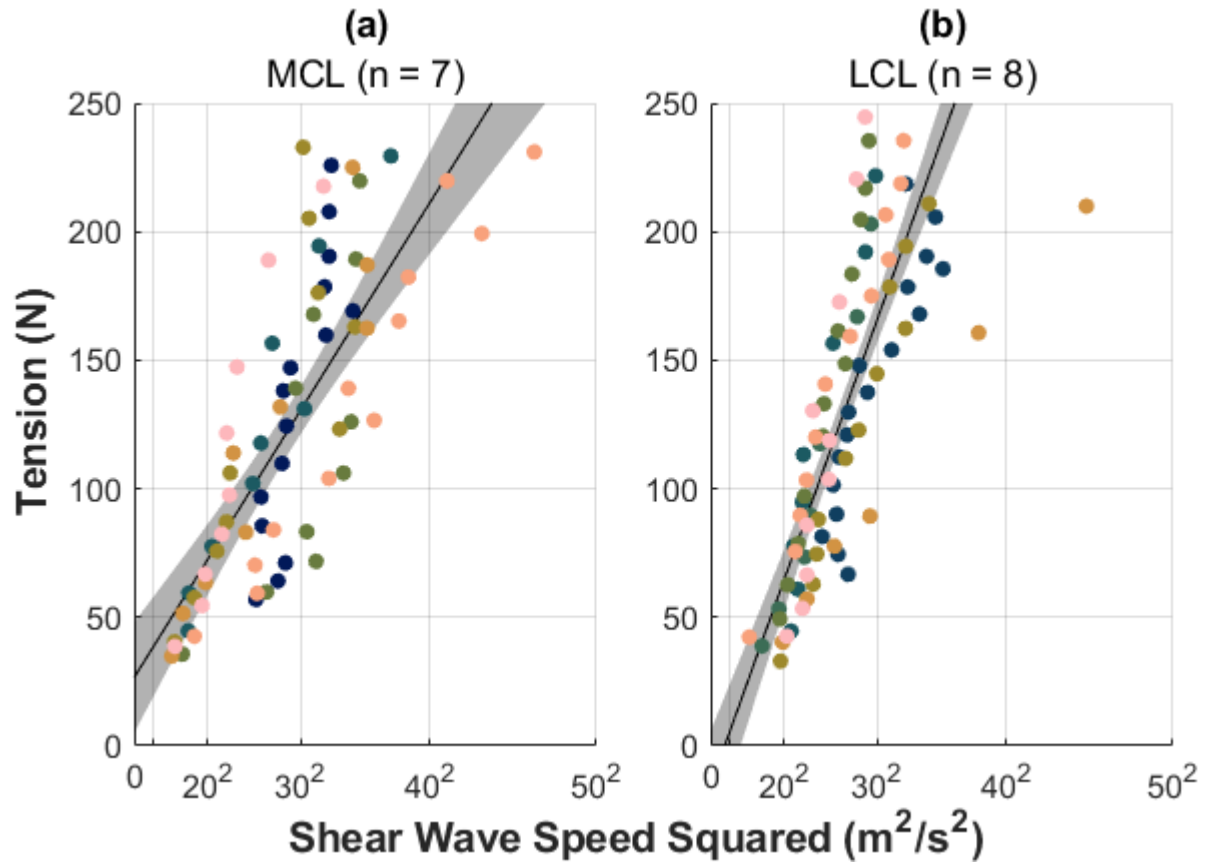


Figure 3. Scatter plots show the linear relationship between shear wave speed squared and tension after incremental releases in both the (a) MCL and (b) LCL. The solid black line indicates the linear regression of the specimens pooled together, and the gray shaded region represents a 95% confidence interval of the fit line. The data points for each specimen are plotted in a different color.⁴³

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Supplement A: Piezoelectric Tapper and Accelerometer Arms

The piezoelectric tapping device consisted of (1) a 3D-printed component (Tough resin, FormLabs, Somerville, MA) (tapper) that rotated about a revolute joint in the tapper tower (Figure 1), (2) a piezoelectric actuator (PK4JQP1, Thorlabs Inc, Newton, NJ) housed in a cavity in the 3D-printed component, and (3) a brass rod with a 3D-printed head (Tough resin, FormLabs, Somerville, MA) to transfer the piezoelectric tapper excitations to the ligament (Figure S-1). The application force of the 3D-printed head against the ligament was controlled by the tension in the fishing line (Dorisea Extreme Braid 0.63 mm) that connected the 3D-printed component to the tapper load cell (Figure 1).

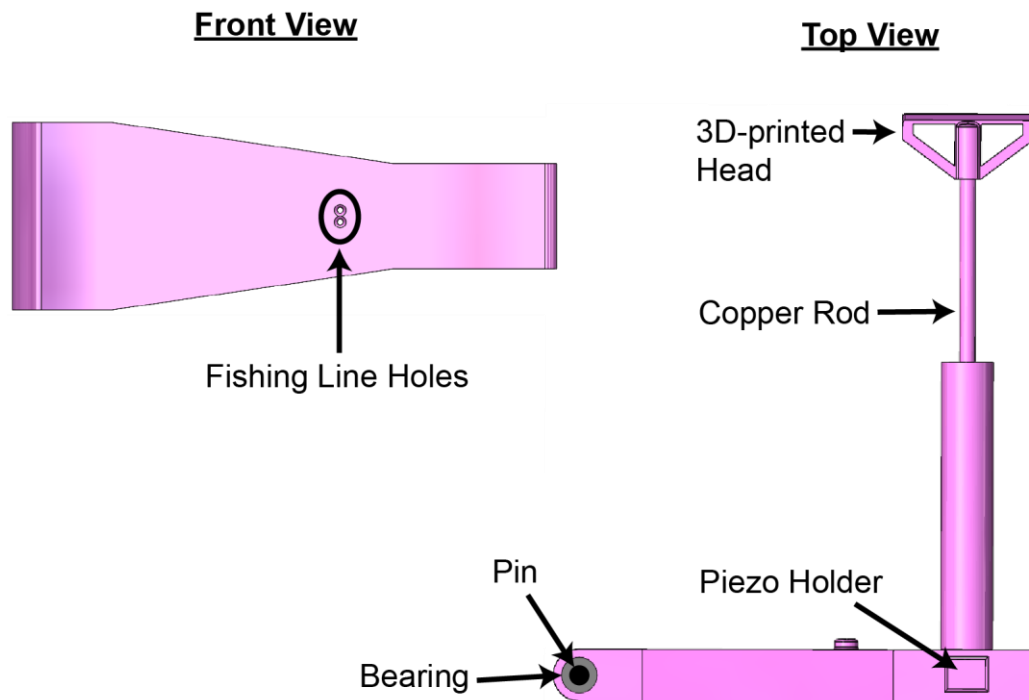


Figure S-1. The piezoelectric tapping device consisted of a 3D-printed component (Tough resin, FormLabs, Somerville, MA) with a cavity to house the piezoelectric actuator. We drilled two holes in the device to tie fishing line through. We used the tension in the fishing line to adjust application force of the 3D-printed head (Tough resin, FormLabs, Somerville, MA) against the ligament. We used a rod with a 3D-printed head to transfer the excitations from the piezoelectric actuator to the ligament.

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The accelerometer arms were 3D-printed components (Tough resin, FormLabs, Somerville, MA), each with a small cavity to house a single-axis accelerometer (Model 352C23, PCB Piezotronics) (Figure S-2). The ends of the accelerometer arms were spaced 6.5 mm apart. Both arms rotated around a pin to increase or decrease the application force of the arm against the ligament. We set this force using fishing line (Dorisea Extreme Braid 0.63 mm) tied through the accelerometer arms. We placed two bearings on each end of each accelerometer arm to reduce friction and minimize transfer of vibrations from one arm to the other.

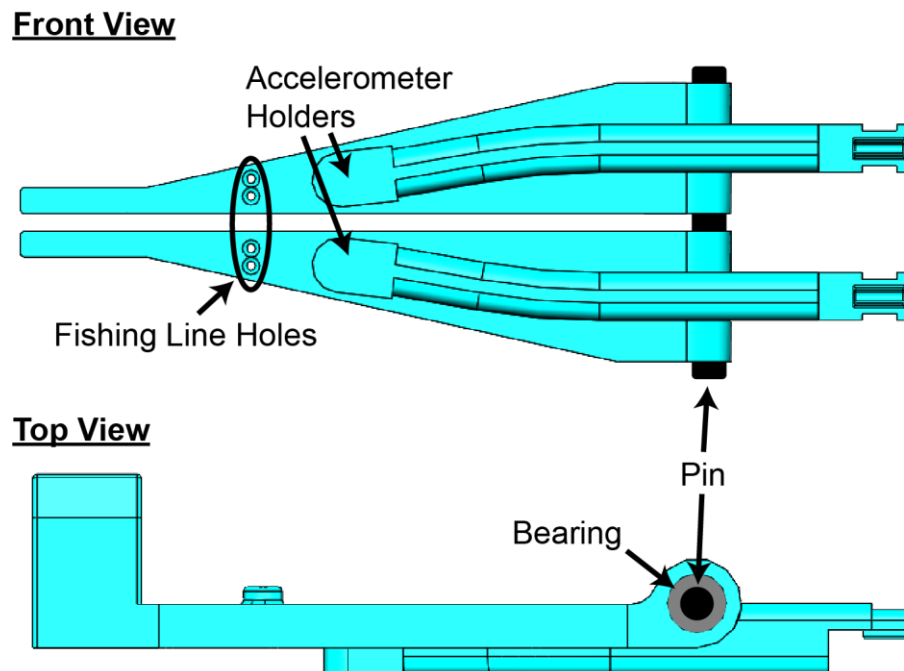


Figure S-2. The accelerometer arms were each 3D-printed with a cavity to house an accelerometer. We drilled two holes in each arm to tie fishing line through. The tension in the fishing line set the application force of each arm against the ligament.

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Supplement B: Repeatability Tests

Day-to-Day Repeatability:

To determine the repeatability of our custom shear wave excitation and measurement system, we measured the shear wave speeds at three different loads prior to releases in a subset of three ligaments. As described in the Methods Section (*Experimental procedure*), we mounted the three components of the tensiometer, which included a piezoelectric tapping device and two accelerometers (Figures 1, S-1, and S-2), in a 3D-printed structure that pressed each component against the ligament with a controlled force of 1.4 ± 0.2 N (Figure S-3). The piezoelectric tapping device induced shear waves in each specimen by delivering 20- μ m impulsive taps across the width of the ligament at tap rate of 10 Hz. The pair of single-axis accelerometers spaced 6.5 mm apart tracked the shear wave propagation.

We first measured the shear wave speed in the ligament under an applied axial load of 250 N. After a shear wave speed measurement, the tension in the fishing line was released to remove contact between the exterior surface of the ligament and the piezoelectric tapper and accelerometer arms. We moved and then replaced the structures clamped to the base of the MTS to mimic possible positioning variability between testing days. After replacement of the structures, we re-tensioned the fishing line attached to the load cells until we restored the application force to the original 1.4 ± 0.2 N. We repeated this process three times for each load (250 N, 150 N, and 50 N) for a total of nine measurements for each ligament.

The repeatability limit, defined in ASTM E177 as “the value below which the absolute difference between two individual test results obtained under repeatability conditions may be expected to occur with a probability of approximately 0.95”,³³ was calculated using Equation S.1:

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$$r = 1.96 * \sqrt{2} * STD \quad (S.1)$$

489 where r is the repeatability limit and STD is the repeatability standard deviation. STD was
490 calculated as the pooled standard deviation of shear wave speed measured in the three trials at
491 each load level for each specimen. The repeatability limits were 6.70 m/s and 4.58 m/s for the
492 MCL and LCL (Figure S-4a and S-4b), respectively.

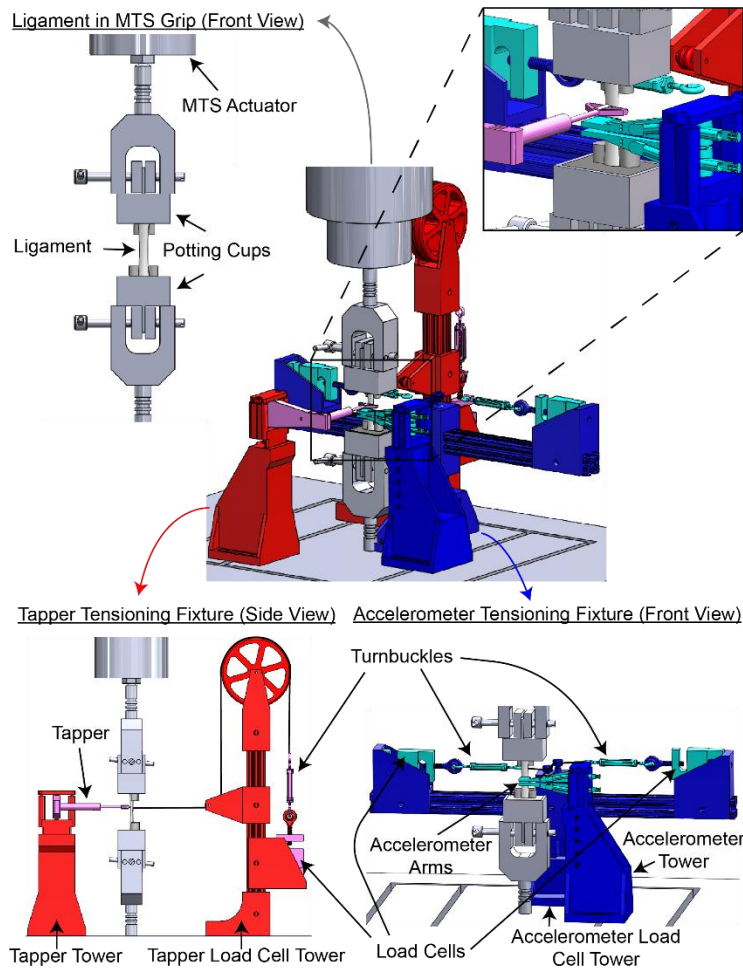


Figure S-3. (Duplication of Figure 1 for convenience). Renderings of CAD assembly show the test apparatus used to control the application forces of the tensiometer components against the ligament (middle). The bony attachments of the ligament were fixed within two potting cups mounted to the MTS (top left). The tensiometer components included two 3D-printed arms holding accelerometers and a tapper (top right). The Tapper Tensioning Fixture (bottom left) included a tapper mounted to the tapper tower and a load cell attached to the tapper load cell tower. The tapper and load cell were connected by a turnbuckle and fishing line (Dorisea Extreme Braid 0.63 mm, not pictured in the middle image for clarity), and the turnbuckle was used to adjust the tension in the fishing line to control the application force of the tapper against the ligament. The tapper, load cell, and turnbuckle are shown in pink, and the tapper tower and tapper load cell tower are shown in red. The Accelerometer Tensioning Fixture (bottom right) included two accelerometer arms mounted to the accelerometer tower and two load cells attached to the accelerometer load cell tower. Each accelerometer arm was connected to its own load cell with a turnbuckle and fishing line. Like in the Tapper Tensioning Fixture, a turnbuckle was used to adjust the tension in each fishing line to control the application force of each accelerometer arm against the ligament. The accelerometer arms,

load cells, and turnbuckles are shown in cyan, and the accelerometer tower and accelerometer load cell tower are shown in blue.

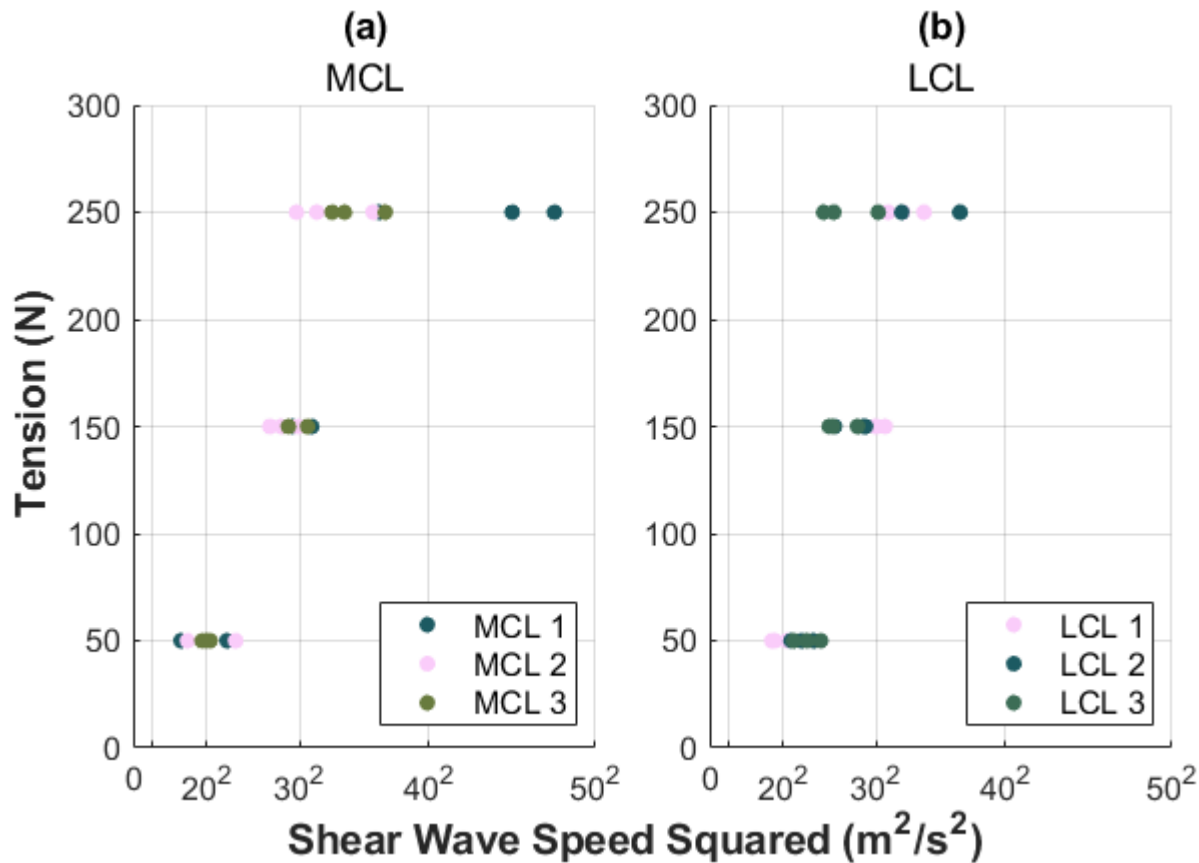


Figure S-4. Scatter plot of tension vs shear wave speed for the day-to-day repeatability study. Each point represents one trial. The repeatability limits were (a) 6.70 m/s for the MCL and (b) 4.58 m/s for the LCL.

Trial-to-Trial Repeatability:

To determine the repeatability of resetting the application force of the accelerometer arms and tapper on the ligament between each measurement, we measured the shear wave speed at the same three loads as in the *Day-to-Day Repeatability* section (i.e., 250 N, 150 N, and 50 N). However, instead of re-positioning the structures, we only loosened the tension in the fishing line before resetting the application forces to the original 1.4 ± 0.2 N.

Similar to the *Day-to-Day Repeatability* section, we calculated the repeatability limit for the MCL and LCL using Equation S.1. The repeatability limits were 1.49 m/s and 2.70 m/s for the MCL and LCL (Figure S-5a and S-5b), respectively.

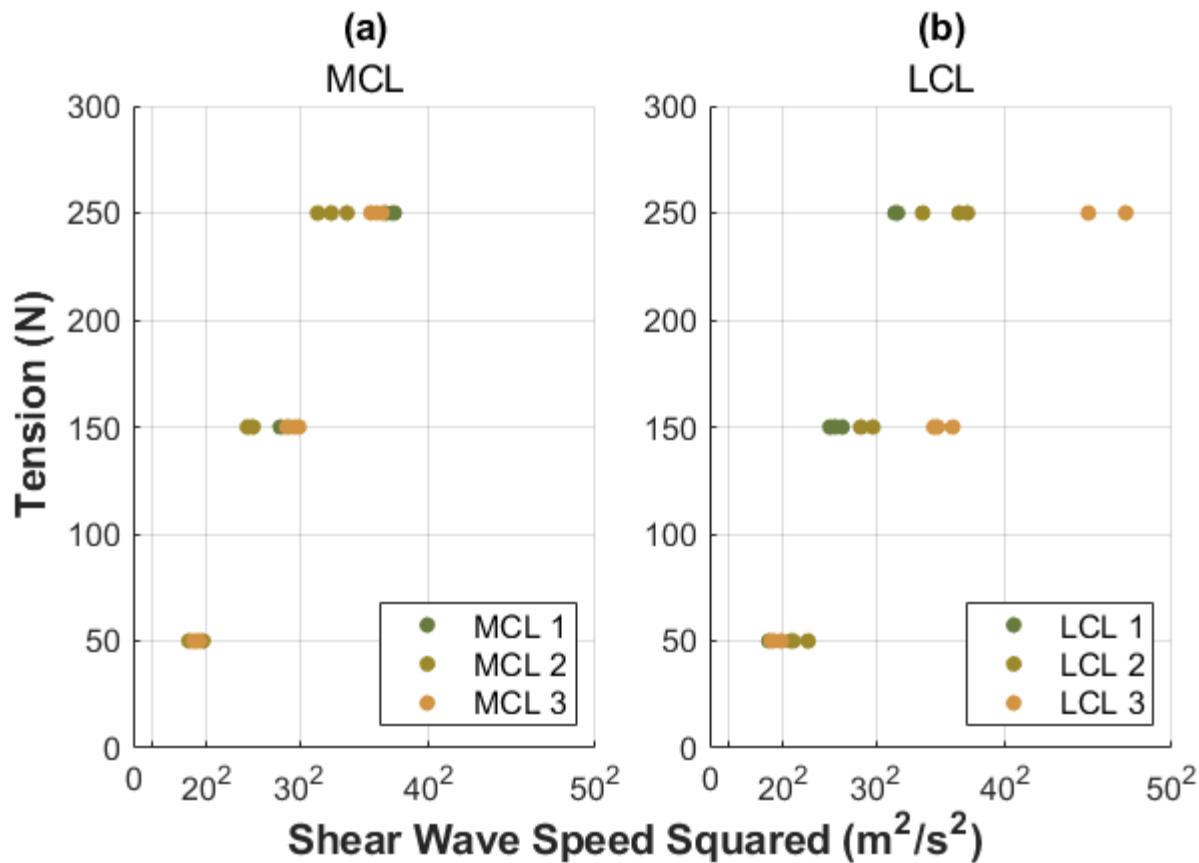


Figure S-5. Scatter plot of tension vs shear wave speed for the trial-to-trial repeatability study. Each point represents one trial. The repeatability limits were (a) 1.49 m/s for the MCL and (b) 2.70 m/s for the LCL.

Supplement C: Individual Ligament Data

The shear wave speed squared and tension measurements collected during the incremental release experiment for each specimen are shown below (Figure S-6 and S-7).

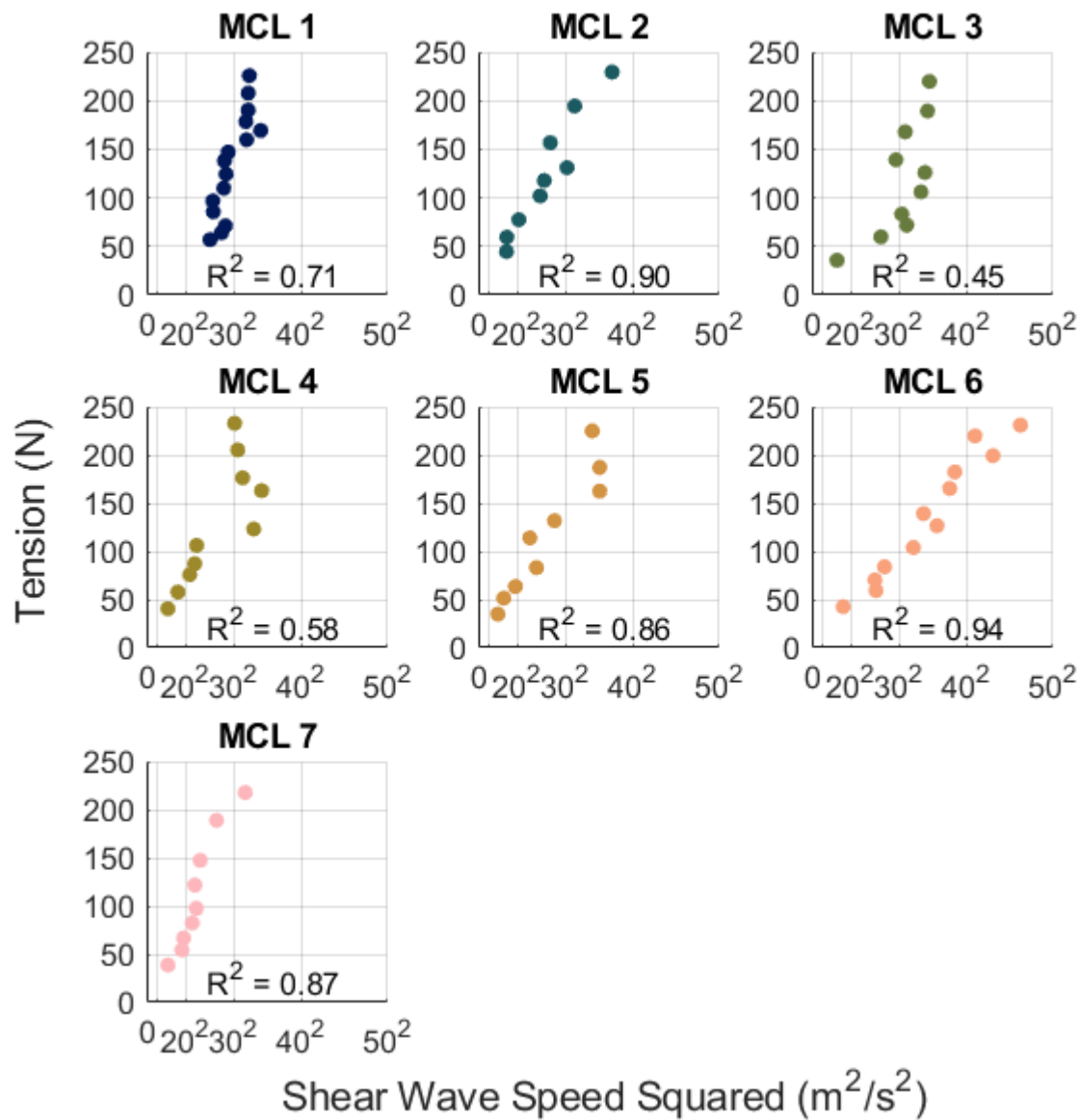


Figure S-6. Scatter plots show the shear wave speed-tension data collected for each of the MCL specimens and the corresponding R^2 -value for a simple linear regression between shear wave speed squared and tension.

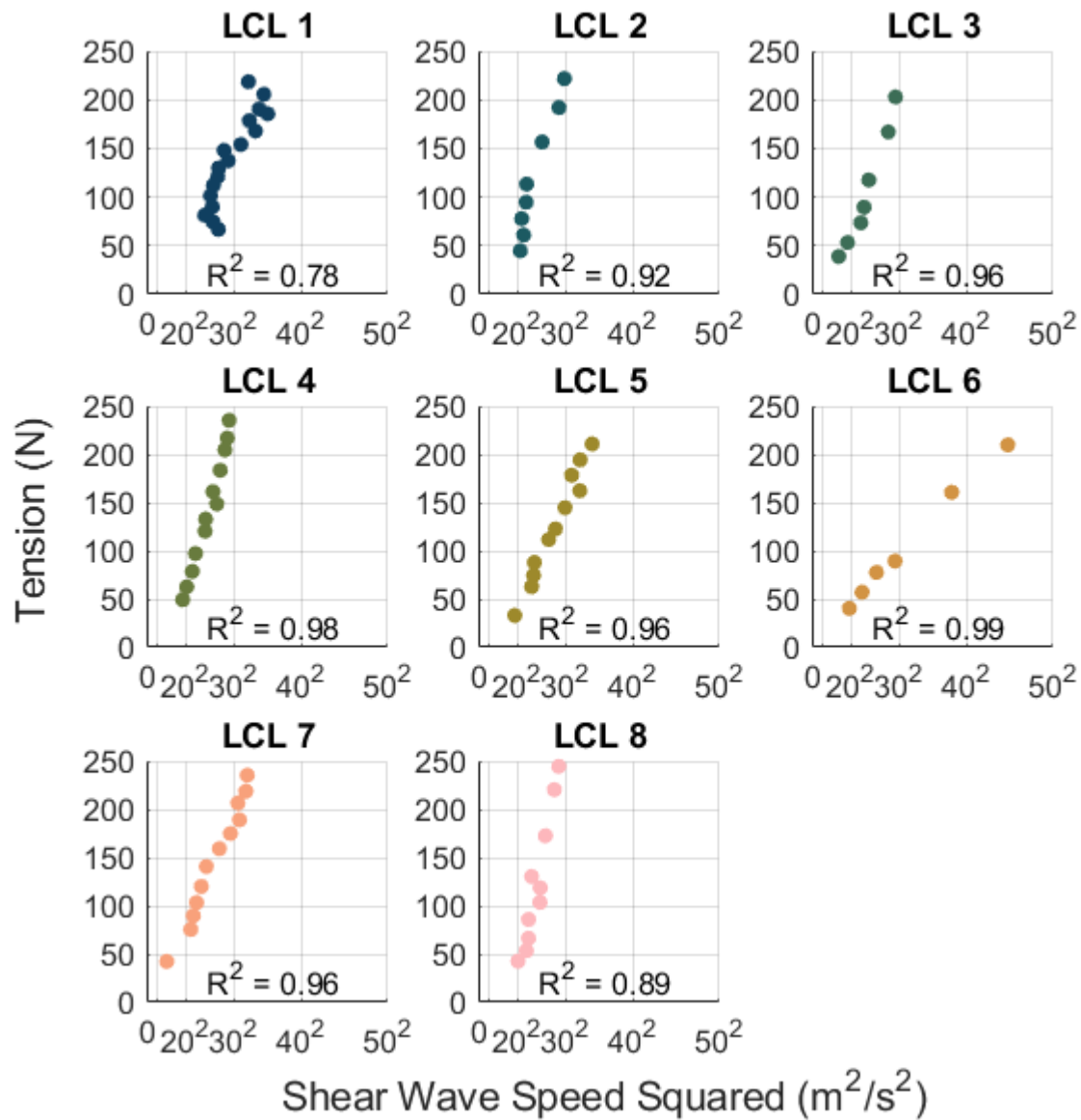


Figure S-7. Scatter plots show the shear wave speed-tension data collected for each of the LCL specimens and the corresponding R^2 -value for a simple linear regression between shear wave speed squared and tension.

Supplement D: Cross-Sectional Images

Following dissection, but prior to our experimental protocol, we used ultrasound imaging to measure cross-sectional areas for each ligament. We mounted specimens in an electrodynamic testing system (Acumen 3, MTS, Eden Prairie, MN) and immersed them in an acoustic tile-lined water bath (Aptflex F28, Precision Acoustics, Dorchester, UK) to facilitate ultrasound imaging (SonixTOUCH Research, BK Medical, Peabody, MA). We pre-loaded each ligament to 10 N of axial tension to remove slack. We collected cross-sectional B-Mode ultrasound images at 0.1 mm increments along the length of the ligament using a linear array ultrasound transducer (L14-5W/38, BK Medical, Peabody, MA) that was affixed to a motorized linear stage (Newmark Systems Inc, Ranch Santa Margarita, CA). The B-Mode imaging was performed through an acoustic window (0.25 mm thick polycarbonate).

We segmented ligament cross-sections from the ultrasound images using an open-source 3D visualization software (3D Slicer, Cambridge, MA). We computed the width, thickness, aspect ratio, and cross-sectional area of each ligament using this software. We measured each of these properties over the middle 5 mm of the ligament to avoid errors due to soft tissue near the insertions. The width was computed as the greatest edge-to-edge distance of the cross-section that intersected the centroid. The thickness was the edge-to-edge distance perpendicular to the width axis and coincident with the centroid. The aspect ratio was computed as the ratio between the width and the thickness of the ligament cross section.

We computed the average MCL to have the following properties: width of 11.5 mm, thickness of 3.4 mm, aspect ratio of 3.0, and area of 32.6 mm² (Table S-1). We computed the average LCL to have the following properties: width of 12.4 mm, thickness of 4.6 mm, aspect ratio of 2.7, and area of 41.0 mm² (Table S-2).

536 **Table S-1.** MCL properties measured using ultrasound imaging and processed with a 3D
537 visualization software.

Specimen	Width (mm)	Thickness (mm)	Aspect Ratio	Area (mm ²)
MCL 1	10.2	3.7	2.7	28.0
MCL 2	12.8	4.0	3.2	36.0
MCL 3	15.0	3.4	4.4	37.4
MCL 4	12.5	4.1	3.1	38.4
MCL 5	12.4	4.4	2.8	41.1
MCL 6	16.7	3.3	5.1	42.2
MCL 7	12.2	4.6	2.6	38.0
Average	11.5	3.4	3.0	32.6

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539 **Table S-2.** LCL properties measured using ultrasound imaging and processed with a 3D
540 visualization software.

Specimen	Width (mm)	Thickness (mm)	Aspect Ratio	Area (mm ²)
LCL 1	13.8	4.9	2.8	46.1
LCL 2	13.0	4.0	3.3	40.8
LCL 3	11.5	3.9	3.0	37.4
LCL 4	12.0	4.1	2.9	37.0
LCL 5	12.4	5.2	2.4	43.6
LCL 6	10.7	4.7	2.3	36.9
LCL 7	12.9	5.1	2.6	46.8
LCL 8	12.8	4.9	2.6	39.8
Average	12.4	4.6	2.7	41.0

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