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The Injury Response of Geriatric Mouse Patellar Tendons

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Abstract

Injury adversely impacts the structure and mechanical properties of a tendon, thus causing pain and disability. Previously, we demonstrated that patellar tendons in mature (P120) and aged (P300) mice do not recover original functionality, even six weeks after injury, and that uninjured geriatric tendons (P570) are functionally inferior to uninjured mature tendons. In this study, we hypothesized that the repair response in injured geriatric mice would be further compromised, thus undermining patellar tendon function post-injury. Patellar tendons from wild-type mice were injured at 540 days. At 3 and 6 weeks post-surgery, structural, mechanical and biochemical analyses were performed and compared to uninjured controls. Mechanical properties of geriatric tendons failed to improve after injury. When compared to mature and aged tendons post-injury, it was determined that at no age was there a suitable repair response. In previous studies, we were able to associate the absence of SLRPs with phenotypic changes both early and late in repair. Here we found that SLRPs were significantly decreased after injury, thus offering a possible explanation for why geriatric tendons were unable to mount an adequate repair response. Thus, we conclude that regardless of age after maturity, tendon healing ultimately results in a substandard outcome.

Keywords

Tendon; Injury; Aging; Small Leucine Rich Repeat Proteoglycans

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Author Contributions Statement: Hypotheses and experimental strategies originated from DEB and LJS. DEB, LJS, MJM, AAD, and MRB were involved in experiment design. Mouse injuries were performed by AAD, MRB, and DPB. Data acquisition and analyses were done by MJM, AAD, MRB, and SMA. Interpretation of data was done by DEB, LJS, MJM, AAD, and MRB. Manuscript preparation was done by MJM, AAD, MRB, DEB, and LJS.

Introduction

The composition and structure of tendons play important roles in their functions. These highly durable fibrous connective tissues are composed of cells within an extracellular matrix rich in collagens, proteoglycans, and water. Tendons have a specialized structure that endows them with specific mechanical and organizational properties allowing for proper transmission of force from muscle to bone. However, when injured, tendons are adversely impacted as tissue damage results in mechanical alterations that affect performance and can lead to substantial pain and disability.[1; 2] Recovery is typically incomplete and results in functional deficits and reductions in activity level.[3; 4]

Aging tendons are at increased risk for injury due to changes in composition, mechanical properties, and structural integrity.[5; 6] In a previous study, we demonstrated that with advancing age, mouse patellar tendons exhibited deteriorating viscoelastic properties between 150 and 570 days of age.[7] These included a significant reduction in dynamic modulus which corresponds to decreased ability to properly transfer force, and also involved a significant increase in the tangent of the phase angle between stress and strain which indicated that the tendons were becoming more dissipative and less able to store and release elastic energy during cyclic loading, thus affecting functionality of the tendon.[7] Additionally, geriatric tendons did not demonstrate significant differences in the expression of small leucine-rich repeat proteoglycans (SLRPs). However, they did exhibit decreased cellularity, altered tenocyte morphology, and reduced collagen fiber alignment.[7] Geriatric patellar tendons were structurally and functionally inferior to mature tendons.

To better understand the effect of age on tendon healing, we followed the repair response at 3 and 6 weeks post-injury in 150 and 270 day-old mice.[8] With age, while there was some slight improvement from the time of injury to 3 weeks, tendons failed to exhibit improved mechanical properties between 3 and 6 weeks post-injury. When mice null for the SLRPs biglycan and decorin were injured, no improvements in biomechanical properties were found over the interrogated 6 weeks of repair.[8] Thus, we showed that aging played a role in tendon repair.

To determine if tendon biomechanical properties further deteriorate when injury occurs with advanced age, we injured wildtype mice patellar tendons at 540 days of age. We hypothesized that compared to mature and aged animals, injured geriatric mice will have an inferior repair response and thus function would be further compromised. At 3 and 6 weeks post-surgery, structural, mechanical and biochemical analyses were performed and compared to uninjured 570 day-old controls.

Materials and Methods

Animals, injury model, and sample collection

C57BL/6 wild-type (WT) female mice were used in this study with IACUC approval. Wild-type female mice were obtained from Jackson Laboratories and housed at the University of Pennsylvania. Mice at 540 days post-natal (n=34) underwent bilateral surgery on their patellar tendons as previously described. [7; 9–11] Briefly, after incising the skin, two incisions

were made along the sides of the tendon to allow for a rubber coated backing to slip under the tendon. Then a 0.75 mm biopsy punch was used to create a full thickness, partial width defect injury in the center of the patellar tendon. Finally, the skin was sutured and the animals were allowed to return to normal cage activity. Animals were sacrificed at 3 (n=15) or 6 (n=19) weeks after surgery. To serve as uninjured controls, 16 additional mice were sacrificed at 570 days of age. Immediately after sacrifice, a randomly selected hind limb (left or right) was removed, wrapped in gauze, soaked in PBS, and frozen for the future mechanical testing. For the contralateral limb, the tendon was bisected through the scar tissue and prepared for gene expression and transmission electron microscopy (TEM). Tendon biomechanical properties, expression levels and TEM findings from these geriatric mice were compared to data from identically injured and uninjured "mature" 120-day old mice (uninjured n=17, 3 weeks n=15, 6 weeks n=12) and "aged" 270-day old mice (uninjured n=14, 3 weeks n=13, 6 weeks n=12) from previous studies that all encompass one large investigation in which surgeries, tissue collections, data acquisition, and data analysis were all done using an identical approach.[7; 8; 12]

Mechanical Testing

Mechanical testing was performed as previously described with the other studies in the project utilizing n=10–14 biological replicates per group.[7; 8; 12] Briefly, tibia-patellar tendon-patella complexes were isolated. Cross-sectional areas of patellar tendons were measured with a custom laser device. The tendon was stamped into a "dog-bone" shape and the cross-sectional area measured again with the same device. Then the tibia was potted in acrylic and secured with a staple; the pot and the patella were gripped with custom fixtures and loaded into a PBS bath on an Instron 5848 universal testing system for mechanical testing. Throughout preparation, the tendon was kept hydrated with PBS. The dynamic testing protocol was comprised of: 1) preconditioning; 2) stress relaxation at strain levels of 4%, 6%, and 8%; 3) a sinusoidal frequency sweep (10 cycles at 0.01, 0.1, 1, 5, and 10 Hz) at each strain level; 4) return to gauge length; and 5) ramp to failure.[7; 8; 10; 12–15] At each strain level and frequency, the dynamic modulus |E*| (the ratio of stress amplitude to strain amplitude) and the tangent of the phase angle between stress and strain (tanδ, a measure of viscoelasticity equal to the ratio of dissipated to stored energy) were computed. At each strain level and frequency, t-tests were performed to compare across injury states. Bonferroni corrections were applied to adjust for multiple comparisons. Significance was set at $p \leq 0.05/2$ and $0.05/2 \leq p \leq 0.10/2$ was considered a trend. Inferences were made based upon consistent results.

RT-qPCR

Expression levels were quantified by real-time quantitative polymerase chain reaction assay (RT-qPCR) for biglycan, decorin, fibromodulin, and lumican relative to beta-actin as previously described.[7; 8; 12] Briefly, individual tissue samples (n=10–15 per group) were homogenized in QIAzol reagent (Qiagen, Valencia, CA). Total RNA was isolated using a RNeasy Micro Kit (Qiagen) as previously adapted.[16; 17] From each individual sample, mRNA from 25 ng of total RNA was reverse transcribed into cDNA and amplified by Single Primer Isothermal Amplification (SPIA) using the WT-Ovation RNA Amplification System (NuGEN, San Carlos, CA)was amplified (SPIA).[7; 8; 12; 18] The resulting SPIA product was

purified with a QIAquick PCR Purification Kit (Qiagen) and the amplified cDNA was eluted with 30 μl RNase-free water.[7; 8; 12] For RT-qPCR analysis, 1 μl of amplified cDNA template was added to a reaction volume of 20 μl per well in an ABI StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA) with a Fast SYBR Green Master Mix (Applied Biosystems). Mouse specific primers for *β-actin* (*Actb*: F—AGATGACCCAGATCATGTTTGAGA; R—CACAGCCTGGATGGCTACGT), *decorin* (*Dcn*: F—GCTGCGGAAATCCGACTTC; R—TTGCCGCCCAGTTCTATGAC), *biglycan* (*Bgn*: F—CCTTCCGCTGCGTTACTGA; R—GCAACCACTGCCTCTACTTCTTATAA), *fibromodulin* (*Fmod*: F—GAAGGGTTGTTACGCAAATGG; R—AGATCACCCCCTAGTCTGGGTTA), and *lumican* (*Lum*: F—TCCACTTCCAAAGTCCCTGCAAGA; R—AAGCCGAGACAGCATCCTCTTTGA) were used. Amplified cDNA for each individual uninjured or injured patellar tendon sample was analyzed in triplicate with a single negative RT control (0.83 ng total RNA per well) for each sample and each gene. Gene specific efficiencies were calculated using LinRegPCR v7.5 software for each qPCR plate and the relative quantity of mRNA for each gene of interest was computed using the relative gene expression ratios formula.[19; 20] Specimens were analyzed per group (n=10–15). Outliers were removed if their measured expression was greater than two standard deviations from the mean; even with such a criterion, at most only 1 outlier per group was identified and eliminated. To test for differences in gene expression, Mann-Whitney tests were used and significance was set at and $p \leq 0.05$ and $0.05 \leq p \leq 0.1$ was considered a trend.

Transmission Electron Microscopy

Tendons were isolated and prepared as previously described.[7; 8; 12] Tendons were fixed with 2.5% glutaraldehyde/4% formaldehyde, post-fixed with osmium tetroxide, serial ethanol dehydrated, embedded in Epon 812 and polymerized at 60 °C. Ultra-thin cross-sections were imaged on JEOL 1400 transmission electron microscope (JEOL Ltd., Tokyo, Japan) equipped with a Gatan Orius widefield side mount CC Digital camera (Gatan Inc., Pleasanton, CA). Tendon fibril diameter analysis was performed as previously described, where pooled data from 5 mice/group with 10 digital images from each tendon/mouse taken at 60,000x were used.[7; 8; 12] Images were analyzed using an RM Biometrics-Bioquant Image Analysis System (Nashville, TN). A region of interest (ROI) of appropriate size was determined for such digital images so that a minimum of 80 fibrils could be measured within each image using 1 or 2 ROIs. Thus, at least 80 fibrils were examined per image, or technical replicate, for 10 images per mouse for each of the 5 mice. Fibril diameters were determined along the minor axis of the fibril cross-section with diameter measurements pooled into groups by injury state.

Consideration of Statistical Power

Power analyses were conducted to understand sample size prior to the start of the comprehensive set of studies on injury, aging, and the influence of SLRPs.[7; 8; 12] To achieve 80% power, it was determined that 10 animals were needed per group for mechanical analyses. Sample sizes for electron microscopy data [7; 8; 12; 16; 21; 22] and gene expression were similarly calculated from previous experiments.[7; 8; 12–14; 16; 23]

Results

SLRP Gene Expression

Expression was interrogated by RT-qPCR in uninjured and injured geriatric patellar tendons. Biglycan expression (Fig. 1A) had a downward negative trend from uninjured to 6 weeks post-injury (5.2-fold reduction) with expression significantly decreased between 3 and 6 weeks post-injury (1.9-fold reduction). Expression levels for decorin and fibromodulin (Fig. 1B,C) decreased significantly between uninjured and 3 weeks post-injury (6.2- and 5.7-fold reductions, respectively) with small but statistically significant increases in expression between 3 and 6 weeks post-injury (2.0- and 1.8-fold increases, respectively). Notwithstanding, overall expression for decorin and fibromodulin (Fig. 1B,C) was significantly decreased for geriatric tendons between uninjured and 6 week post-injury groups (3.1- and 3.2-fold decreases, respectively). Expression of lumican (Fig. 1D) has a slight downward trend across group.

Biomechanical Properties – Geriatric Tendons

The biomechanical properties of the geriatric tendons were compared between uninjured and injured states. Reported here are findings at 4% and 8% strains; findings at 6% strains were similar to those at 8% (data not shown). At strains of 4% and 8%, significant decreases in dynamic modulus (|E*|) were noted when comparing uninjured P570 tendons to tendons 3 weeks post-injury (Fig. 2A). Additionally, at strains of 4% and 8%, significant increases in dynamic modulus (|E*|) were noted when comparing geriatric tendons at 3 weeks and 6 weeks post-injury (Fig. 2A); however, for both strains, dynamic modulus did not differ between uninjured tendons and those 6 weeks post-injury. Moreover, at strains of 4% and 8%, significant increases in viscous dissipation (tanδ) were noted when comparing uninjured P570 tendons to tendons 3 weeks post-injury and significant decreases were noted when comparing geriatric tendons at 3 weeks and 6 weeks post-injury (Fig. 2B); however, for both strains, there were no differences in viscoelasticity between uninjured tendons and those 6 weeks post-injury. Cross-sectional areas of the P570 patellar tendons were significantly increased at 3 weeks post-injury relative to both uninjured and 6 weeks post-injury; cross sectional areas 6 weeks post-injury were only slightly greater than that of uninjured tendon (Fig 2C) While findings indicate significant differences when essentially comparing start and endpoints to the healing midpoint in this study, it is important to compare these findings to previous the biomechanical parameters described in the previous studies of mature and aged tendons for the complete investigation of the effects of aging and injury on murine patellar tendons.[7; 8; 12]

Comparative Analysis of Biomechanical Properties Post-Injury: Mature, Aged, Geriatric

To put the findings for the geriatric patellar tendons into perspective, biomechanical parameters of dynamic modulus and viscoelasticity were compared across age – mature (P150), aged (P300), and geriatric (P570) – for each injury state by incorporating these data with data from previous studies.[7; 8; 12] At both 4% and 8% strain levels, dynamic modulus |E*| of the patellar tendon decreased significantly between P150 to P300, between P300 to P570, and overall from P150 to P570 (Fig. 3A). At 4% strain, viscoelasticity (tanδ) increased significantly from P150 to P300, from P300 to P570, and overall from P150 to P570 (Fig.

3D); results were similar for 8% strain. The cross-sectional areas of uninjured P150 tendons were significantly smaller when compared to uninjured P300 and P570 tendons (Fig 3G). Relative to the uninjured states, |E*| is comparatively lower and tanδ is higher at all ages, respectively, as should be noted by review of the y-axis in each panel of Fig. 3. By 6 weeks post-injury, all healing patellar tendons regardless of age ultimately demonstrate no difference for either |E*| (Fig. 3C) or tanδ (Fig. 3F). It should be noted that cross-sectional areas of P150, P300, and P570 tendons are statistically equivalent at 3 weeks post-injury (Fig. 3H), though at 6 weeks post-injury mature and geriatric have similar cross sectional areas (Fig 3I).

Geriatric Tendon Fibril Structure Post-Injury

TEM analysis of fibril diameter distribution for uninjured (Fig. 4A), 3 weeks-post injury (Fig. 4B), and 6 week post-injury (Fig. 4C) geriatric (P570) patellar tendons showed that after injury geriatric tendons had increased numbers of small diameter fibrils after 3 weeks. At 6 weeks post-injury, even greater numbers of smaller diameter fibrils still persisted.

Comparative Analysis of Fibril Structure

Again, to put the findings for the geriatric patellar tendons into perspective, fibril diameter analyses were compared across age – mature (P150), aged (P300), and geriatric (P570) – for each injury state by incorporating these data with data from previous studies (Table 1).[7; 8; 12] In uninjured patellar tendons, mean fibril diameter increased 13.6% between P150 and P300; mean fibril diameter at P570 is within 3% of P300 mean fibril diameter. After injury, mean fibril diameters of the aged and geriatric patellar tendons were greater than the mature tendon at three weeks post-injury (P150: 62.5%; P300: 77%; P570: 79% – percentage relative to uninjured mean diameters at each age). Moreover, at 6 weeks post-injury, mature and aged tendon displayed increases in mean fibril diameter, while mean fibril diameter decreased for geriatric tendon.

Discussion

Though we hypothesized that injured geriatric mice will have an inferior repair response with further compromised function compared to mature and aged animals, this study demonstrated that despite age-related differences among uninjured tendons of the three age groups for biomechanical parameters dynamic modulus and viscoelasticity, there were no significant differences in injured tendons by 6 weeks into healing across the three age groups – mature (P150), aged (P300), and geriatric (P570). Detailed findings of the changes in these parameters in uninjured patellar tendons with aging have been previously reported.[7] Interestingly, the values of the biomechanical parameters dynamic modulus and viscoelasticity in the injured geriatric tendons were roughly equal to those in the mature and aged injured group, as well as those in the uninjured geriatric group. Thus, tendons post-injury have a decreased ability to transfer force and decreased dynamic modulus of aged tendons therefore indicates less resistance to strain and are more likely to dissipate energy as opposed to storing it. This indicates that functionality of post-injury repair tissue at any age is approximately as sound as that of uninjured geriatric tendon.

Our results demonstrate that the inferior repair outcomes appear to be independent of age. For all three age groups at 3 weeks post-injury the cross-sectional area of the tendon was greater than that of the uninjured tendon and the tendon at 6 weeks post-injury; this is likely due to the presence of the repair callus in the tendon mid-repair.[24] Moreover, the biomechanical results corroborate previous studies that also have examined tendon function post-injury at different ages.[25–27] Furthermore, in previous studies, we have demonstrated a role for SLRPs in the repair response.[8; 12] Without the earlier and later contributions of biglycan and decorin, respectively, in repair, tendon healing was impaired.[8] In this study, decreases in biglycan, decorin, and fibromodulin suggest that suitable levels of these SLRPs were not achieved and thus offer one reason why maturation of geriatric tendon fibrils post-injury was either not observed or might be delayed. Three “snapshots” or measurements of expression were considered to gauge what was happening with tendon repair in this study. We saw more variation of SLRP expression in the uninjured tendons. The variation could be explained by housekeeping gene stability or sample purity. However, in comparison to our previous studies, similar levels of variation have been seen for these SLRPs, though notably statistically significant gene expression differences were still found. [7; 8; 12; 28] Interestingly, the early and late repair responses in the geriatric patellar tendon demonstrate less variability. This could be because SLRP expression is significantly reduced in these injured tendons and a certain level of expression is required to maintain synthesis of these matrix assembly regulatory proteins. Thus, reduced levels of these SLRPs could impact repair and affect biomechanical outcomes in geriatric tendons.

This study was not without limitations. Structural, mechanical and gene expression measurement were not conducted until three weeks after surgery, and these measurements were only taken up to six weeks. Therefore, the initial repair response of tendon was not assessed, and long term results were not measured that might clarify a delay versus the failure to achieve increases in mean geriatric tendon fibril diameters associated with fibril maturation. Moreover, instead of comparing injured tendons to tendons receiving sham surgeries, we used uninjured tendons as a control. Also, in this study, histological analyses are not reported. Additionally, the actual protein content for SLRPs in the injured tendons was not assessed due to the limited amount of tendon sample; therefore, post-translational regulation of SLRPs was not taken into account. Yet, for the SLRPs, gene expression and protein content have been shown to be correlated in this model.[29] Furthermore, we used a single housekeeping gene for this study as with the other studies encompassing the comprehensive project; nonetheless, in interpreting findings we followed the same consistent strategy so that these expression data, along with the mechanical and ultrastructural data, all provide support to our conclusions.[7; 8; 12]

It should be noted that gross observation of ambulation of injured mice of all ages and at each time point, though not formally measured, demonstrated no more than very minimal behavioral changes, even immediately after surgery. Mice appeared able to ambulate normally within several minutes of waking from anesthesia. Speculative points include that prey animals have evolved to minimize gait changes after injury which would make them more vulnerable and that the murine experience of pain is dramatically different from that of humans. These mice were also not challenged to perform activities such as treadmill

running, though increased activity has been shown to exacerbate symptoms of injury in other studies and could have in the injured mice as well.[30; 31]

This study provides insight into the effect of aging on the repair response to injury at any age in the tendon. This study of geriatric tendons along with previous studies of mature and aged patellar tendons all collectively demonstrate that at any age, healing of a tendon injury ultimately produces a substandard outcome. This finding is relevant to both clinical care and basic science efforts to improve tendon healing. Future work will investigate how molecular constituents within the matrix might be manipulated to understand the mechanisms governing the findings presented here and in consideration of future interventions that could improve tendon repair.

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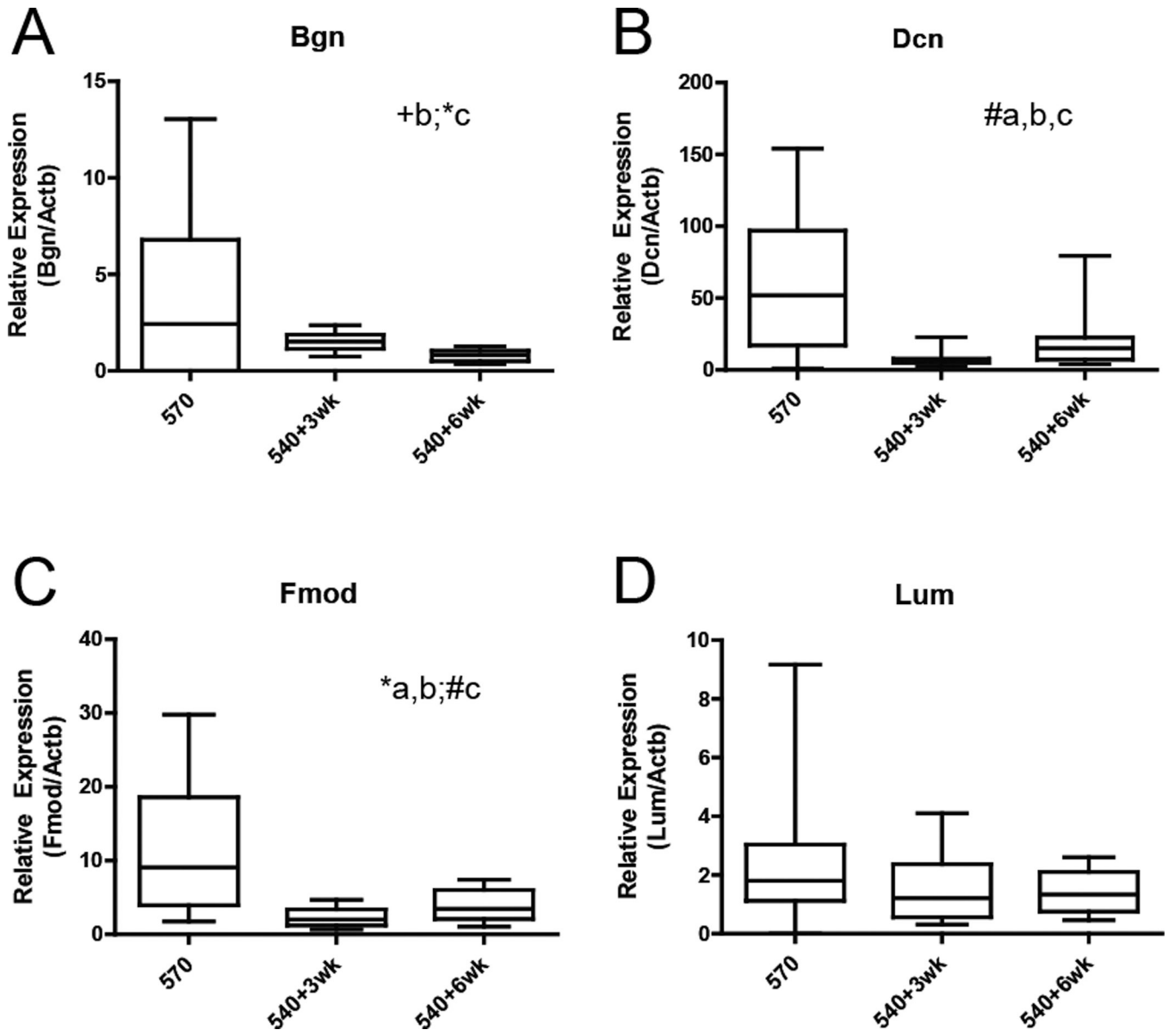


Fig. 1. Gene Expression of SLRPs in Geratric Tendons after Injury

(A) Expression of *Bgn* trended downward at 3 weeks and 6 weeks post-injury, relative to an uninjured state, with a significant decrease seen between 3 and 6 weeks post-injury. Expression of *Dcn* **(B)** and *Fmod* **(C)** significantly decreased post-injury when comparing uninjured to 3 weeks and 6 weeks, though a slight but significant rebound was seen between 3 and 6 weeks for both SLRPs. (D) There was no change in *Lum* expression across injury state. Box and whisker plots give minimum and maximum range, median (vertical bar), and first and third quartiles for each group. Significance was determined by Mann-Whitney Wilcoxon Test, two-tailed with levels of significance described as a combination of **a** (uninjured vs. 3 weeks), **b** (uninjured vs. 6 weeks), or **c** (3 weeks vs. 6 weeks), and * ($p < 0.05$) and # ($0.05 < p < 0.10$).

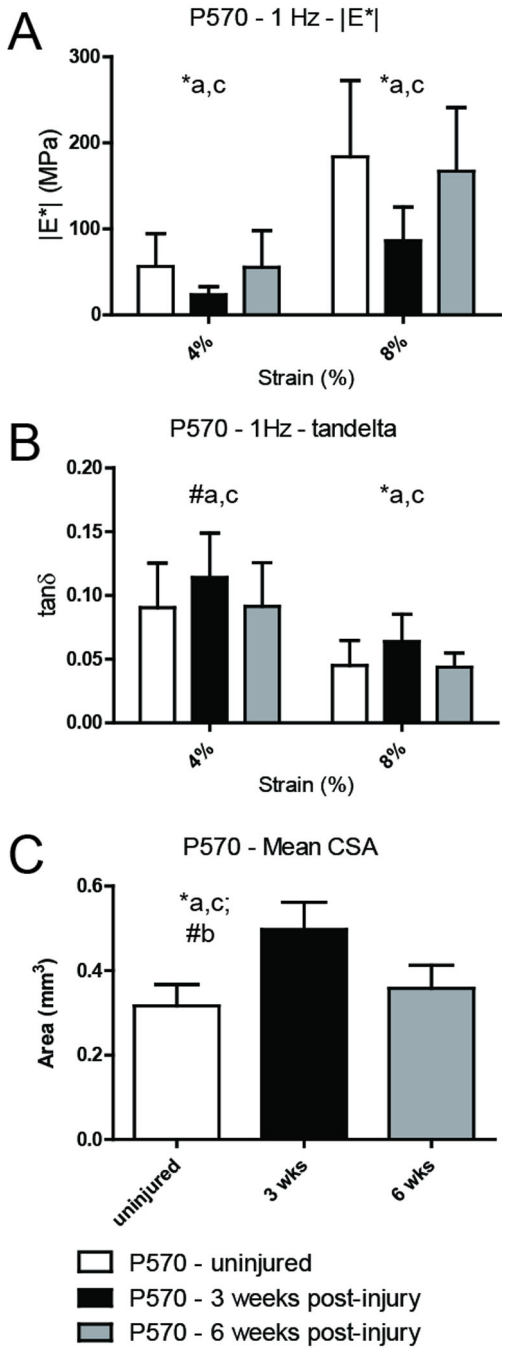


Fig. 2. Biomechanical Properties of Geriatric Tendons after Injury

Dynamic modulus **(A)** and viscoelasticity, tangent of the phase angle **(B)**, were examined between uninjured and injured states in geriatric patellar tendons. In performing biomechanical analyses, cross-sectional area of the tendons were also measured **(C)**. **(A)** At strains of 4% and 8%, significant decreases in dynamic modulus (|E*|) were noted when comparing uninjured P570 tendons to tendons 3 weeks post-injury; in contrast, significant increases in dynamic modulus (|E*|) were noted when comparing geriatric tendons at 3 weeks and 6 weeks post-injury. Dynamic modulus did not differ between uninjured tendons

and those 6 weeks post-injury. **(B)** At strains of 4% and 8%, significant increases in viscoelasticity (tanδ) were noted when comparing uninjured P570 tendons to tendons 3 weeks post-injury and significant decreases were noted when comparing geriatric tendons at 3 weeks and 6 weeks post-injury; however, there were no differences in viscoelasticity between uninjured tendons and those 6 weeks post-injury. **(C)** Cross-sectional areas of geriatric tendons increased significantly 3 weeks post-injury, but by 6 weeks post-injury mean cross-sectional area is only slightly increased, relative to uninjured geriatric tendon. Measurements depicted are means ± standard deviations. Significance is described as a combination of **a** (uninjured vs. 3 weeks), **b** (uninjured vs. 6 weeks), or c (3 weeks vs. 6 weeks), and * ($p < 0.05/2$) and # ($0.05/2 < p < 0.10/2$).

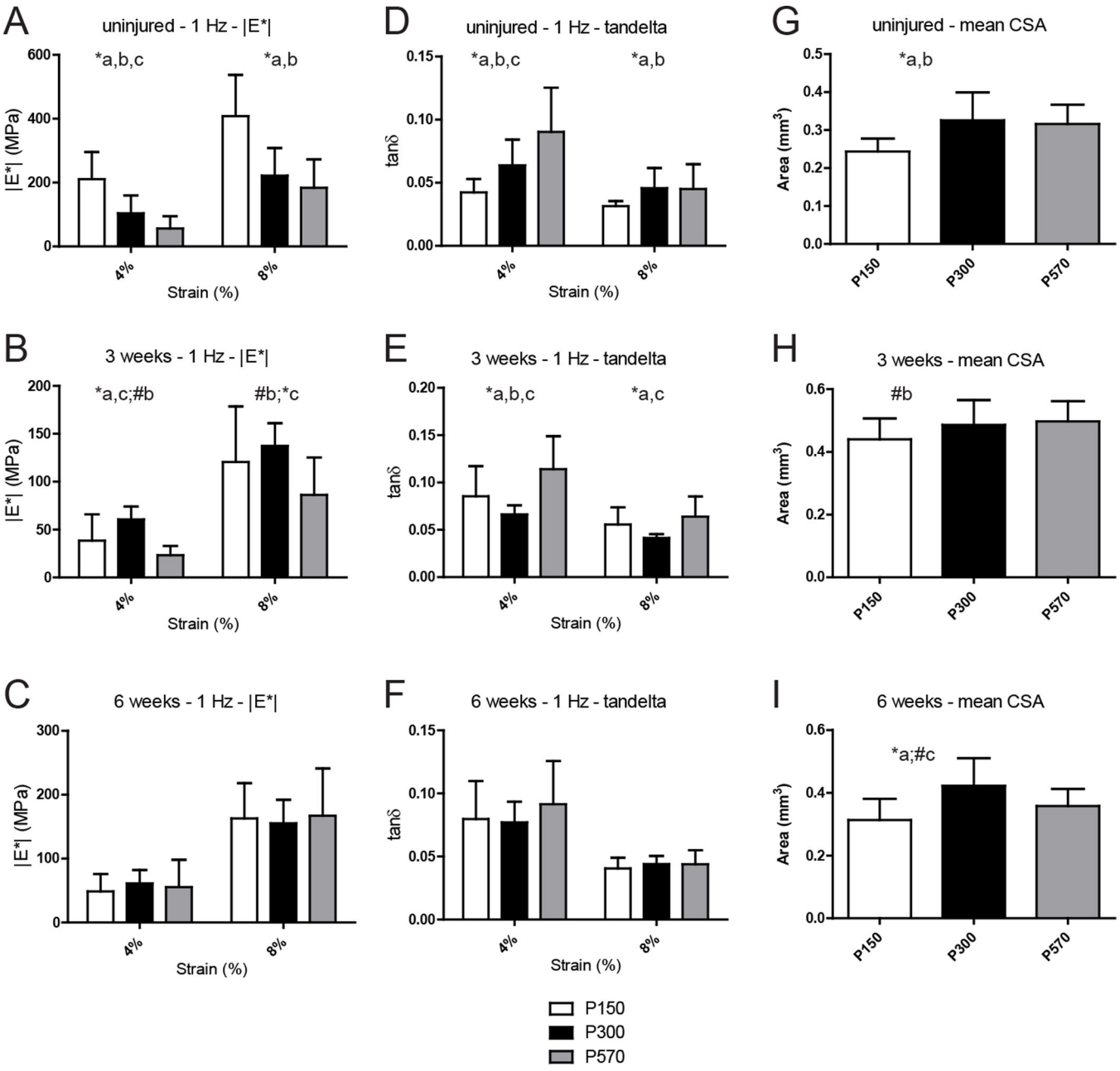
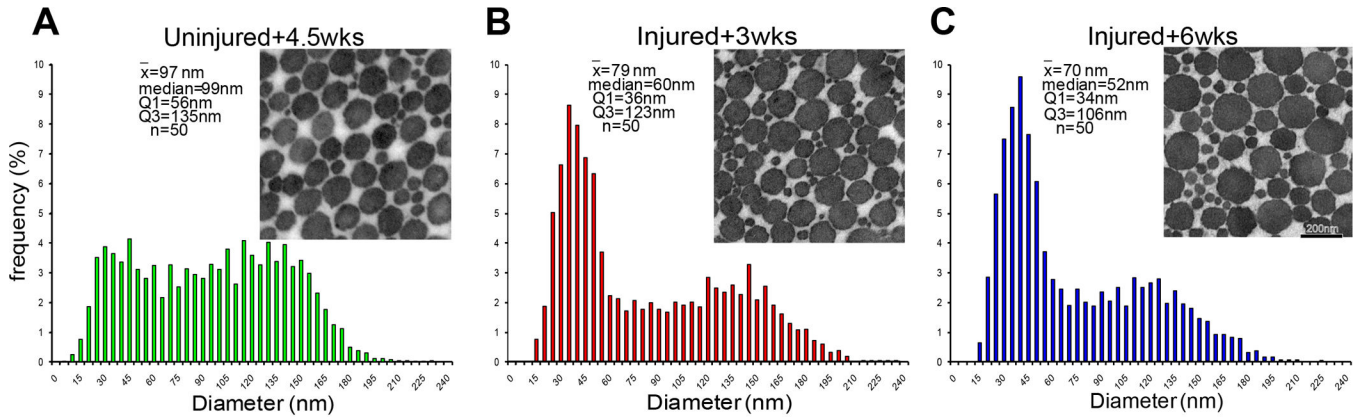


Fig. 3. Comparative Analysis of Biomechanical Properties Across Age and Injury State

Dynamic modulus **(A-C)**, viscoelasticity **(D-F)**, and cross-sectional areas **(G-I)** were compared for mature (P150), aged (P300), and geriatric (P570) tendons at the uninjured, 3 weeks post-injury, and 6 weeks post-injury states. Strains are reported and 4% and 8% at 1 Hz frequency. **(A)** At 4% strain level, dynamic modulus |E*| of the patellar tendon decreased significantly between P150 to P300 and again between P300 to P570. **(D)** Viscoelasticity (tanδ) increased significantly between P150 to P300 and again between P300 to P570. Results were similar for 8% strains **(A, D)**. At 3 weeks post-injury, |E*| is comparatively greater and tanδ is relatively lower in aged patellar tendons, relative to mature and geriatric tendons **(B, E)**. However, relative to the uninjured states, respectively, |E*| is comparatively

lower and tanδ is higher at all ages. By 6 weeks post-injury, all healing patellar tendons regardless of age ultimately demonstrate no difference for either |E*| **(C)** or tanδ **(F)**. Mean cross-sectional area of uninjured patellar tendons is greater for P300 and P570 mice, relative to P150 mice **(G)**; however, at 3 weeks post-injury mean cross-sectional areas are increased relative to uninjured tendons yet nearly equivalent across the age groups **(H)**. At 6 weeks post-injury, mean cross-sectional area of patellar tendons is slightly greater for P300, relative to P150 **(I)**. Measurements depicted are means ± standard deviations. Significance is described as a combination of **a** (150 days vs. 300 days), **b** (150 days vs. 570 days), or **c** (300 days vs. 570 days), and * ($p < 0.05/2$) and # ($0.05/2 < p < 0.10/2$).



Old age (540+)	Q1	Q2	Q3	Mean	sd (n=50)
540d+4.5wks	55.5	99.4	134.9	97.1	10.8
540d+3wks	36.1	60.2	123.4	79.2	17.8
540d+6wks	34.4	51.5	105.9	70.3	11.3

Fig. 4. Geriatric Tendon Fibril Structure Post-Injury

TEM analysis of fibril diameter distribution (n=4–6 per injury state) for uninjured **(A)**, 3 weeks-post injury **(B)**, and 6 week post-injury geriatric (P570) patellar tendons **(C)**. After injury of geriatric tendons, increased numbers of small diameter fibrils are notable at 3 weeks with even greater numbers of smaller diameter fibrils still persisting at 6 weeks post-injury.

Table 1

Comparison of Patellar Tendon Fibril Diameters, by Age and Injury State

	150 days			300 days			570 days		
	uninj	**3 wk**	**6 wk**	**uninj**	**3 wk**	**6 wk**	**uninj**	**3 wk**	**6 wk**
Mean	88	55	59	100	77	78	97	79	70
Median	91	40	42	104	61	59	99	60	52
Q1	61	29	29	70	37	38	56	36	34
Q3	114	68	85	131	115	115	135	123	106

Tendon fibril diameter measurement values are in nm. Uninjured and injured tendon data for 150 day old tendons were previous reported.[7,12] Uninjured and injured tendon data for 300 day old tendons also were previously reported.[8] Uninjured tendon data for 570 day old tendons were previously reported.[7]