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The Role of the Non-Collagenous Extracellular Matrix in Tendon and Ligament Mechanical Behavior: A Review

Tendon is a connective tissue that transmits loads from muscle to bone, while ligament is a similar tissue that stabilizes joint articulation by connecting bone to bone. The 70–90% of tendon and ligament's extracellular matrix (ECM) is composed of a hierarchical collagen structure that provides resistance to deformation primarily in the fiber direction, and the remaining fraction consists of a variety of non-collagenous proteins, proteoglycans, and glycosaminoglycans (GAGs) whose mechanical roles are not well characterized. ECM constituents such as elastin, the proteoglycans decorin, biglycan, lumican, fibromodulin, lubricin, and aggrecan and their associated GAGs, and cartilage oligomeric matrix protein (COMP) have been suggested to contribute to tendon and ligament's characteristic quasi-static and viscoelastic mechanical behavior in tension, shear, and compression. The purpose of this review is to summarize existing literature regarding the contribution of the non-collagenous ECM to tendon and ligament mechanics, and to highlight key gaps in knowledge that future studies may address. Using insights from theoretical mechanics and biology, we discuss the role of the non-collagenous ECM in quasi-static and viscoelastic tensile, compressive, and shear behavior in the fiber direction and orthogonal to the fiber direction. We also address the efficacy of tools that are commonly used to assess these relationships, including enzymatic degradation, mouse knockout models, and computational models. Further work in this field will foster a better understanding of tendon and ligament damage and healing as well as inform strategies for tissue repair and regeneration. [DOI: 10.1115/1.4053086]

Introduction

Tendon is a commonly injured connective tissue that transmits loads from muscle to bone, whereas ligament is a structurally and compositionally similar tissue that stabilizes joint articulation by connecting bone to bone. High incidence rates of tendinopathy, a degenerative condition characterized by pain, swelling, and tenderness, are reported among both athletic and nonathletic populations [1]; one study suggests upwards of two million cases of lower extremity tendinopathy occur in the U.S. per year [2]. Ligament sprains and tears are also pervasive, with an estimated 224,000 anterior cruciate ligament (ACL) tears occurring in the U.S. per year [3]. These pathologies have previously been associated with altered loading environments or repetitive overloading, emphasizing the importance of understanding the mechanical behavior of tendon and ligament [4–6]. Furthermore, because tendon and ligament's mechanical responses are largely driven by their microstructures, a better understanding of the structure–function relationship between tissue composition and multiscale mechanical behavior is needed in order to understand injury risk, progression, and healing and to improve tissue repair and replacement strategies. Tendon and ligament are also known to differ in functional demands, injury modality, and bulk mechanical behavior (Fig. 1(*b*)) [11], which warrants further research in order to determine how microstructural composition contributes to their unique macroscale responses.

The majority of tendon and ligament consist of an organized hierarchical collagen structure (Fig. 2), with the remaining fraction composed of non-collagenous constituents. The 70–90% of tendon and ligament's dry weight consists of collagen I, an extracellular matrix (ECM) protein which is primarily aligned along the tissue's long axis [11,16,17]. At the nanoscale level, individual collagen molecules form a triple helix structure known as tropocollagen. Several grouped tropocollagen molecules form microfibrils, which then aggregate to form collagen fibrils. Covalent crosslinks also form orthogonally to adjacent microfibrils, contributing to mechanical stiffness in the fiber direction [18]. Next, collagen fibrils group to form larger fibers, which in turn group to form fiber bundles encased in a loose connective sheath called the epitenon. Some tendons and ligaments consist of a single fiber bundle, while others consist of multiple fiber bundles, or fascicles [19]. Adjacent fascicles are connected by an interfascicular matrix (IFM), also known as the endotenon. At the outermost level, the entire tissue is encased in another loose connective sheath called the paratenon, which contain blood vessels. Outside of the collagen I hierarchy, the remaining 10–30% of the ECM consists of minor collagens and a variety of non-collagenous constituents such as proteoglycans, glycosaminoglycans (GAGs), and other glycoproteins. These

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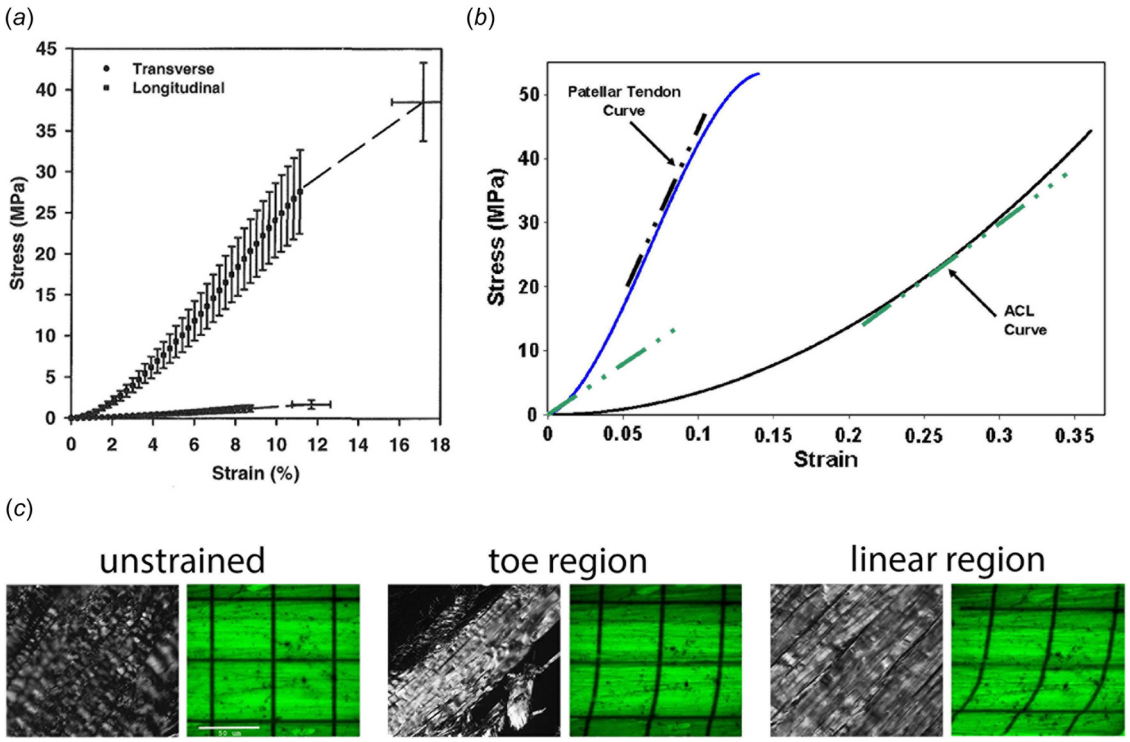

Fig. 1 Quasi-static mechanical behavior of tendon and ligament and the macro- and microscale. (*a*) Representative stress–strain curves for human MCL strained at a rate of 10 mm/s, demonstrating the high degree of anisotropy observed in ligament and tendon. Linear moduli during longitudinal (axial) and transverse tensile tests were reported to be 332.15±58.27 MPa and 11.02±3.57 MPa, respectively (Adapted with permission from Quapp and Weiss [7]. Copyright 1998 by American Society of Mechanical Engineers). (*b*) Representative stress–strain curves and tangent lines (used to calculate toe and linear moduli) for the human ACL and patellar tendon strained at a rate of 100%/s. Linear moduli for ACL and patellar tendon in males were reported to be 128±25 MPa and 479±141 MPa, respectively (Adapted with permission from Chandrashekar et al. [8]. Copyright 2008 by Elsevier). (*c*) Collagen fibrils uncrimp in the toe region (Adapted with permission from Miller et al. [9]. Copyright 2012 by Elsevier), while fibril sliding, demonstrated by shearing of a grid photobleached onto the ECM (Adapted with permission from Cheng and Screen [10]. Copyright 2007 by Springer Nature), is the predominant mechanism of tissue deformation at high strains in the linear region.

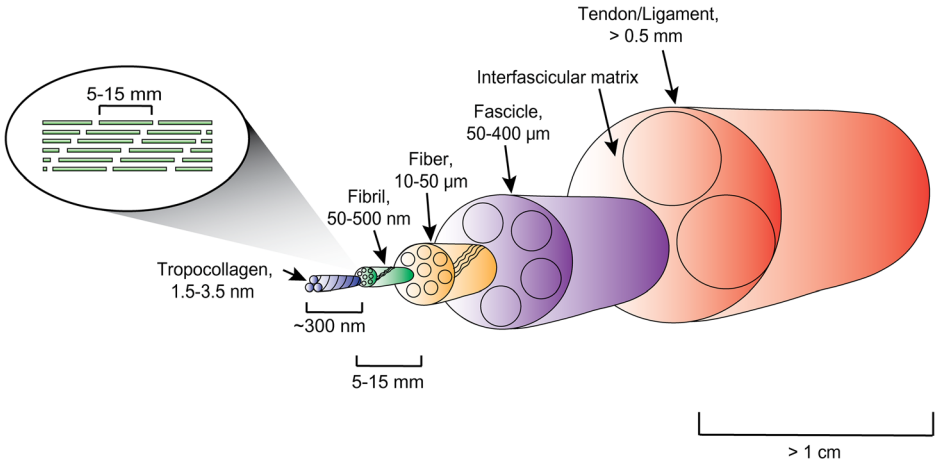

Fig. 2 Diagram of hierarchical tendon and ligament structure with representative diameter [12] and length [13,14] scales for human tissue, as first proposed by Kastelic et al. [15]. Fibril discontinuity is demonstrated throughout the length of the tissue.

constituents make up the interfibrillar and interfascicular matrices that connect adjacent collagen fibrils and fascicles, respectively.

Together, the collagenous and non-collagenous portions of the ECM govern tendon and ligament's multiscale mechanical behavior. At the fibril and fiber levels, the collagen structure exhibits a characteristic wavy crimp pattern and provides tendon and ligament with highly nonlinear and anisotropic mechanical properties (Fig. 1(*a*)) by supporting the majority of loading in the fiber direction (axial). Specifically, the stress–strain curves of tendon and ligament exhibit a characteristic nonlinear "toe" region in which collagen uncrimps and a "linear" region in which collagen is fully uncrimped. Additionally, in both viscoelastic and

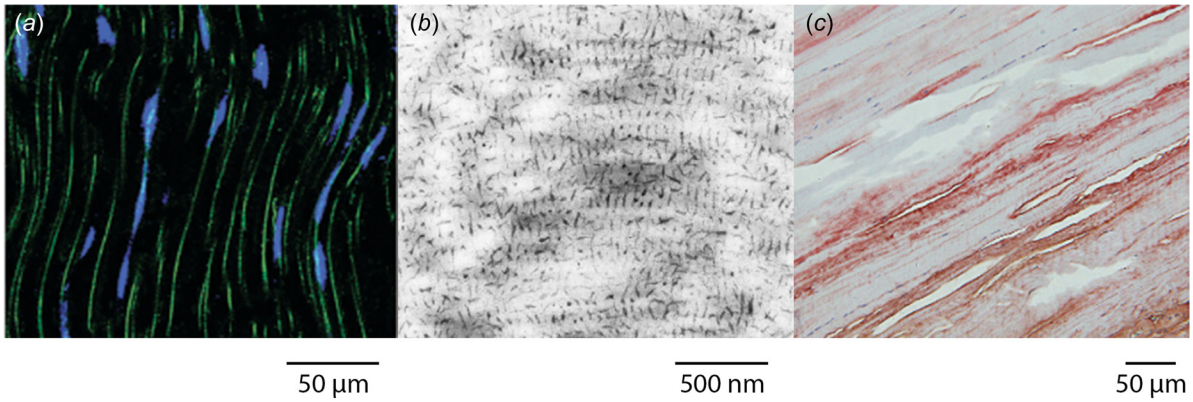

Fig. 3 Staining of mature tendon to visualize elastin, the proteoglycan-GAG bridge, and lubricin. (*a*) Immunostaining of elastin, which exhibits a wavy crimp pattern along the fiber direction (vertical), surrounding tenocyte nuclei (Adapted with permission from Grant et al. [47]. Copyright 2013 by Wiley). (*b*) Cupromeronic blue staining of proteoglycans, which reveals that the proteoglycan–GAG bridge is oriented orthogonally to horizontal collagen fibrils (Adapted with permission from Liao and Vesely [48]. Copyright 2007 by Elsevier). (*c*) Immunostaining of lubricin in which intense staining is observed between collagen fascicles (running along the diagonal) and less intense staining is observed within collagen fascicles (Adapted with permission from Sun et al. [49]. Copyright 2015 by Wiley). Images were obtained from bovine flexor tendon, porcine mitral valve chordae tendinae, and human Achilles tendon, respectively.

quasi-static axial loading regimes, tendon and ligament exhibit sliding of collagen fibrils and fascicles past one another in conjunction with shear deformation of the interfibrillar and interfascicular matrices (Fig. 1(*c*)) [10,20–23]. While this behavior suggests that the interfibrillar and interfascicular matrices facilitate load transfer between adjacent fibrils and fascicles, the specific ECM constituents involved in this process are heavily debated [24–27]. Shear loading at the macroscale also leads to interfibrillar and interfascicular sliding [28], yet the involvement of the non-collagenous ECM in this response is only partially elucidated [29–31]. Finally, further evaluation is necessary in order to identify the role of the non-collagenous ECM in fiber direction quasi-static mechanical properties, such as tensile modulus, and viscoelastic properties, such as stress relaxation and energy loss, in mature and developing tendons and ligaments. Taken together, it is evident that much work remains to be done in order to completely define the structure–function relationship between non-collagenous ECM constituents and tendon and ligament's macroscale mechanical responses.

Likewise, defining the mechanical function of the tendon and ligament microstructure is crucial to our understanding of multiscale mechanotransduction in homeostasis and disease. From the macro- to microscale, tendon and ligament experience stresses and strains that are transmitted from the tissue-level to the matrix-level to the cell- and nuclear-levels [32,33], resulting in mechanosensitive gene expression that dictates biological outcomes [34]. Non-collagenous proteins that reside in the pericellular matrix, a specialized region of the ECM surrounding cells [35], may be particularly important in this process. From the micro- to macroscale, mechanically stimulated gene expression modulates the dynamic structure and composition of tendon and ligament's non-collagenous ECM [36–38], which in turn may affect the tissue's mechanical behavior across all length scales, further emphasizing the intimate relationship between tissue mechanics, ECM remodeling, and homeostasis. Thus, the non-collagenous ECM plays a key role in driving mechanical behavior across all length scales, not only by providing direct mechanical support but also by facilitating multiscale mechanotransduction which in turn modulates tissue structure and function. For an in-depth discussion of mechanotransduction and homeostasis in tendon and ligament, we refer the reader to several excellent reviews dedicated to this topic [39–42].

The purpose of this review is to discuss existing research regarding the role of the non-collagenous ECM in governing tendon and ligament mechanical behavior. Specifically, we present literature that highlights the role of various non-collagenous ECM constituents in tendon and ligament's quasi-static and viscoelastic responses to tension, shear, and compressive loading in the fiber direction and orthogonal to the fiber direction (transverse). We also discuss the strengths and weaknesses of several methods used to assess these properties, which highlights the need for future studies to clarify existing discrepancies in the literature. By doing so, we aim to move the field toward a complete understanding of the non-collagenous ECM's role in tendon and ligament's micro- and macroscale mechanical behavior.

Elastin

Elastin is a highly extensible matrix protein present at multiple hierarchical levels. It can withstand strains of up to 100% without plastically deforming, which allows it to facilitate elastic recoil and fatigue resistance [43]. As such, elastin has been found at higher concentrations in energy storing tendons that undergo frequent cyclic loading compared to positional tendons that act as structural support for the skeleton [44]. in vivo, elastin resides within elastic fibers, which consist of a desmosine-crosslinked elastin core surrounded by a microfibril scaffold composed of fibrillin 1, fibrillin 2, or both [45]. Elastic fibers have diameters ranging from 200 to 800 nm [46], similar to those of collagen fibrils and fibers, and conform to the pattern of collagen's crimp (Fig. 3(*a*)) [47]. These elastic fibers are present at lower concentrations within collagen fibers and at higher concentrations surrounding tenocytes and within the IFM [47].

Elastin likely plays a role in quasi-static fiber direction mechanics by resisting deformation in response to fiber direction loading. Studies in this area most commonly degrade elastin within the tissue using the enzyme elastase and compare the mechanical behavior of control and elastin-depleted tissues. Notably, elastase-treated tendon and ligament exhibited decreased fiber direction tensile moduli in the toe [50] and linear [51] regions. However, loss of elastin has been associated with an increase in collagen crimp wavelength in the toe region [52], suggesting a concurrent increase in toe region mechanical properties according to the worm-like chain model [53]. While others have hypothesized that elastin primarily contributes to tendon and ligament mechanics by stabilizing the collagen crimp waveform [50], the observed decrease in mechanical properties despite the increase in crimp wavelength in elastin-depleted tissue indicates that elastin's primary mechanical role is more likely to directly bear fiber direction

loads and resist deformation. Additionally, while one study found that the Achilles tendon and supraspinatus tendon (SST) from mice heterozygous for the elastin allele (haploinsufficient) exhibited a 14% increase in fiber direction linear stiffness compared to wild type tendons [54], developmental effects observed in the mouse model are likely responsible for the discrepancy between this study and those discussed previously. In total, these data suggest that elastin's ability to recoil may provide some structural support to the collagen crimp waveform, but its primary purpose is to resist deformation in the fiber direction.

Recent evidence suggests that elastin is a key regulator of energy storage in tendon and ligament, but not other fiber direction viscoelastic behavior. Unexpectedly, older works showed no effect of elastin depletion on hysteresis [50,52] despite elastin's known elastic recoil behavior. Two studies that did find increased hysteresis in elastase-treated tissue did not perform any preconditioning prior to testing, which likely resulted in increased hysteresis and nonreproducible mechanical behavior in both control and elastase-treated samples [51,55]. However, a recent breakthrough study by Godinho et al. showed that elastin depletion increased hysteresis in equine tendon fascicles connected by the IFM, but not tendon fascicles alone [31]. Similarly, elastin depletion drastically decreased the fascicle–IFM–fascicle unit's fatigue life. These findings may explain why Grant et al. reported that elastin depletion did not affect hysteresis, as their study depleted elastin in singular tendon fascicles [52]. Further, Godinho et al. [31] measured hysteresis at a cyclic strain rate 20 and 40 times greater than those used by Henninger et al. [50] and Grant et al. [52], respectively, likely contributing to the observed differences in time-dependent behavior. With regards to other fiber direction viscoelastic properties, Achilles tendons and SSTs from mice haploinsufficient for elastin did not display any differences in peak tensile stress, equilibrium tensile stress, or stress relaxation compared to tendons from wild type mice. Overall, elastin's contribution to fiber direction viscoelastic behavior is largely in line with expectations, as elastin prevents energy loss and contributes minimally to other time-dependent properties.

Finally, studies indicate that elastin plays a large role in tendon and ligament's quasi-static and viscoelastic shear and transverse tensile behavior. Removal of elastin from the medial collateral ligament (MCL) decreased peak stresses by 62% and 70% during cyclic shear and transverse tensile loading, respectively, suggesting that elastin governs a disproportionately large fraction of nonaxial mechanical stress despite only accounting for 4% of the MCL's dry weight [30]. One study also showed that elastase-treated human SST exhibited decreased peak and equilibrium shear stresses during stress relaxation tests at 8% and 16% shear strain, but not 24% shear strain [56]. This study was hindered by a large amount of error at higher strains, which could explain why no significant differences in properties were reported at 24% strain. Finally, the previously mentioned study by Godinho et al. demonstrated that elastin depletion resulted in decreased failure load, decreased stiffness, and increased fascicle sliding in fascicle–IFM–fascicle units subject to shearing of the IFM [31]. Together, these studies suggest that elastin is involved in both quasi-static and viscoelastic responses to nonaxial loading and may play a key role in governing shear behavior of the non-collagenous matrix. However, the mechanism by which elastin contributes to nonaxial mechanical behavior, especially in the transverse direction, is unknown. Future studies in this area would contribute to increased understanding of structure–function relationships in tissues that are subject to significant nonaxial loading, such as the Achilles tendon.

While much progress has been made in defining elastin's mechanical role, off-target proteolytic activity of elastase has been a major limitation of elastin degradation studies. Several studies have reported that elastase treatment significantly decreased GAG content [30,31,52], which makes it impossible to isolate the mechanical contribution of elastin if GAGs also play a role in tendon and ligament mechanics. One study attempted to overcome this limitation by demonstrating that GAG depletion did not affect any of the mechanical properties evaluated with respect to elastin [31]. However, because the mechanical contribution of GAGs is still heavily debated (as will be discussed later in this review), it is difficult to draw definitive conclusions about the role of elastin in mechanics from these studies. Any number of factors may have affected the proteolytic activity of elastase in these studies, including the tissue source, the enzyme source, concentration, and purity, and the length of sample incubation. Interestingly, Fang and Lake employed a higher enzyme concentration and longer incubation time than Henninger et al., yet Henninger et al. reported significant off-target GAG depletion [30] and Fang and Lake did not [56]. Given that these works used samples from human SSTs and porcine MCLs, respectively, there may well be a large organism- and tissue-specific effect of elastase on off-target GAG depletion.

Proteoglycans and Glycosaminoglycans

Proteoglycans are a diverse group of ECM proteins that comprise 1–5% of tendon and ligament's dry weight [57]. They perform numerous critical functions in these tissues, including regulating fibrillogenesis, modulating cell growth, and stimulating immune responses [58]; in fact, the role of proteoglycans in tissue development is much better characterized than the role of proteoglycans in mature tendon and ligament [59,60]. Each proteoglycan has a specific set of GAG side-chains that it binds to, which is typically a combination of dermatan sulfate (DS), chondroitin sulfate (CS), heparan sulfate, keratan sulfate (KS), and the nonsulfated GAG hyaluronic acid. GAGs are highly hydrophilic and therefore play a role in both fiber direction and transverse compression mechanics by modulating tissue water content [17,61]. However, the role of proteoglycans and GAGs in fiber direction tensile, transverse tensile, and shear mechanics is debated. Prior work suggests that small proteoglycans such as decorin, biglycan, lumican, and fibromodulin are present at higher concentrations in midsubstance regions of tendon that are subject mostly to tension, whereas large proteoglycans such as aggrecan and versican are present at higher concentrations in fibrocartilaginous regions of tendon that are subject mostly to transverse compression [17]. This provides some initial context as to the role that each proteoglycan might play in mechanics, although specific structure–function relationships have not been fully elucidated.

Decorin and Biglycan. Decorin is a small leucine-rich proteoglycan (SLRP) that makes up ~80% of the proteoglycan content in tendon and ligament, whereas biglycan is a similar SLRP present in lesser amounts [57]. Both proteoglycans have a sickle-shaped structure that allows them to bind to the exterior of collagen fibrils near the D-period, a dark band that appears every 68 nm along the fibril length [48,62]. Additionally, decorin typically binds to one CS or DS GAG chain, while biglycan binds to two GAG chains, which can be any combination of CS and DS. These GAG side-chains can bind together in interfibrillar spaces due to van der Waals forces, dipole–dipole interactions, hydrogen bonding, and hydrophobic interactions [63], forming a complete proteoglycan–GAG–GAG–proteoglycan bridge (hereafter referred to as the proteoglycan–GAG bridge) that links adjacent collagen fibrils (Fig. 4). As we will discuss in detail here, the proteoglycan–GAG bridge has been hypothesized to facilitate load transfer between adjacent collagen fibrils, although existing evidence suggests that the proteoglycan–GAG bridge may play a minor role at most in tendon and ligament mechanics. However, decorin and biglycan may indirectly contribute to tendon and ligament mechanics by modulating fibril diameter size.

Computational and constitutive models have been used to attempt to demonstrate that it is mechanically feasible for the proteoglycan–GAG bridge to transfer loads between neighboring collagen fibrils. One such computational model simulated molecular mechanics of interactions between CS and collagen to

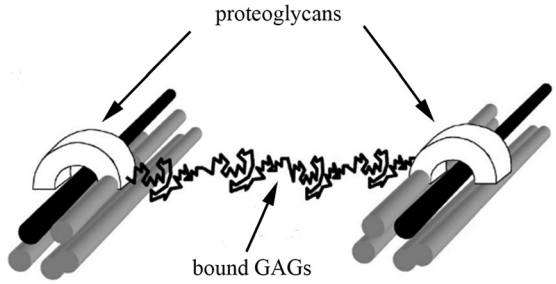

Fig. 4 Proteoglycan molecules (modeled as white semi-cylinders) bound to collagen fibrils (modeled as black rods) that are surrounded by other collagen fibrils (modeled as gray rods) to form a collagen fiber. The proteoglycans are linked via the interaction of two GAGs in the interfibrillar space, forming an interfibrillar bridge. Image adapted with permission from Vesentini et al. [64]. Copyright 2005 by Elsevier.

demonstrate that computed bulk tissue stresses were consistent with experimental findings; the authors of the study suggested that this implicates the proteoglycan–GAG bridge in facilitating load transfer between adjacent fibrils [65]. Another work expanded upon this study by using a poroelastic model to demonstrate that fiber direction tensile strain is transferred through the matrix via extension of the proteoglycan–GAG bridge in conjunction with fibril sliding [66]. Finally, Ciarletta et al. presented a pseudohyperelastic mathematical model that described the contribution of softening effects and breakage and reformation of proteoglycan– collagen bonds to bulk tissue mechanics; this model agreed well with experimental data and implicated the proteoglycan–GAG bridge in contributing to tendon and ligament's viscous response [67].

However, computational models are restricted in their ability to accurately describe mechanical phenomena, which could mean that they overestimate the role of the proteoglycan–GAG bridge in tendon and ligament's mechanical response. Simplification is a key issue among this group of studies, as models have often treated collagen as a solid rod and neglected to account for fibril and fiber uncrimping and reorganization during loading [24,68]. Some studies have also simplified the structural complexity of the proteoglycan–GAG bridge by representing it as a GAG attached directly to the fibrils rather than a dumbbell-like bridge composed of two proteoglycans and a GAG [65]. These simplifications may result in discrepancies between computational and experimental results; one study found no difference in mechanical properties after depleting DS and CS with chondroitinase ABC, but a 14% decrease in linear stiffness after depleting GAGs in a finite element model [68]. Finally, and most importantly, these phenomenological models are limited in their applicability because they are often based on assumptions rather than experimental evidence. For example, the model presented by Ciarletta et al. [67] is limited by a lack of physiological evidence describing the breakage and reformation of covalent bonds as having mechanical significance. Likewise, the GAG–GAG interaction in the proteoglycan–GAG bridge has been computationally modeled as a covalent bond [65], rather than a weak electrostatic interaction [63], which overestimates the stiffness of interconnected GAG chains by several orders of magnitude [69]. These weaker bridges may not be able to withstand physiologic loads as predicted by the computational models described here. In total, because there is significant room to improve computational models of shear load transfer, there is reason to believe that the proteoglycan–GAG bridge may contribute minimally to load transfer between collagen fibrils.

In further support of a load transfer mechanism that is not facilitated by the proteoglycan–GAG bridge, in vitro enzymatic GAG depletion studies have shown that the proteoglycan–GAG bridge has minor influence at most on viscoelastic mechanics and little to no influence on quasi-static mechanics in both fiber direction tension and shear (Table 1). Several studies have shown that depletion of CS and DS from tendon and ligament using the enzyme chondroitinase ABC did not affect peak tensile stresses or fiber direction tensile modulus [26,71,72]. Depletion of DS also did not alter peak shear stresses or shear modulus in ligament [70]. With respect to viscoelasticity, studies have found that GAG depletion does not alter stress relaxation in fiber direction tension [71] or shear [29], while another study only found significant differences in fiber direction tensile stress relaxation after 300 s of relaxation [73]. The study that found differences in relaxation after 300 s was the only study out of this group to fit their data to Fung's quasi-linear viscoelastic model [80], which revealed that removal of CS and DS from bovine digital extensor tendon fascicles decreased fast stress relaxation and increased slow stress relaxation [73]. These findings seem to indicate that comparing time constants is a more robust method of describing changes in tendon and ligament stress relaxation behavior because dynamic stress behavior throughout the testing period, rather than only peak and equilibrium stress, is considered. Similarly, Fang and Lake found no changes in stress relaxation but nonsignificant trends ($p < 0.1$) toward decreased peak and equilibrium shear stresses during stress relaxation of GAG-depleted human SST [29]. Together, these studies demonstrate that although GAG depletion does not seem to alter the percentage of stress relaxation observed in fiber direction tension or shear, noticeable changes to the shape of the stress-time curve may still occur, suggesting a minor role for the proteoglycan–GAG bridge in modulating viscoelastic behavior.

Mouse knockout studies do not clearly support a role for the proteoglycan–GAG bridge in load transfer, although they seem to suggest that decorin is at least involved in fiber direction viscoelastic behavior. Some knockout studies have implicated decorin in stress relaxation behavior and strain rate sensitivity, but not quasi-static or failure properties [74,76,81]. Other studies have attempted to differentiate between the roles of decorin and biglycan across multiple tendons. One such study revealed that decorin knockout increased modulus and stress relaxation in the patellar tendon but not the flexor digitorum longus (FDL) tendon, whereas biglycan knockout decreased peak stress and modulus in the FDL tendon but not the patellar tendon [81]. Another study demonstrated that removing biglycan from the patellar tendon did not affect quasi-static fiber direction properties such as toe modulus, linear modulus, or stress relaxation, but did increase dynamic modulus, a measure of viscoelasticity [78]. In addition, the decorin- and biglycan-null patellar tendon displayed decreased failure stress and stiffness, increased viscous behavior and stress relaxation, and no changes in linear modulus [79]. Interestingly, this is the only mouse knockout study to use an inducible knockout model that removed the influence of decorin and biglycan in mature, but not developing, mice. The fact that these results differ from the results of other mouse knockout studies indicates that the role of decorin and biglycan could change throughout the mouse's life cycle, which agrees with other studies that analyzed the effect of decorin and/or biglycan knockout across age groups [75,77,82]. These results may also confirm a possible synergistic effect in which knockout of both proteoglycans together has more impact than knockout of either decorin or biglycan alone, which has been reported previously in bone [83]. Overall, these mouse knockout studies implicate decorin in limiting tendon and ligament's stress relaxation behavior but do not directly demonstrate decorin's role in quasi-static mechanics. Additionally, decorin and biglycan's mechanical contributions appear to be tendon-specific, although more work is needed to determine which tissues and properties they affect and why their mechanical involvement is not universal. Finally, there is evidence that decorin and biglycan's influence on mechanics is age-dependent, and future work is needed to fully define the mechanical properties that are differentially regulated by decorin and biglycan throughout the development and aging process.

Although the mechanical role of the proteoglycan–GAG bridge is still debated, a large breadth of mouse knockout studies has implicated decorin and biglycan in regulating tendon and

Table 1 Summary of the impact of enzymatic GAG degradation and decorin and/or biglycan mouse knockout (K/O) on tendon quasi-static and viscoelastic material properties during fiber direction tension

Publication	Tissue	Treatment	Impact on quasi-static mechanics	Impact on viscoelastic mechanics
Lujan et al., 2007 [70]	Human MCL	Chondroitinase B	– Peak stress, linear modulus	– Hysteresis
Fessel and Snedeker, 2009 [25]	Rat tail tendon fascicles	Chondroitinase ABC	N/A	– Storage modulus, loss modulus
Lujan et al., 2009 [71]	Human MCL	Chondroitinase ABC	N/A	– Stress relaxation, dynamic modulus, phase shift, peak stress
Rigozzi et al., 2009 [72]	Murine Achilles tendon	Chondroitinase ABC	– Ultimate stress, linear modulus, failure stress	N/A
Svensson et al., 2011 [26]	Human patellar tendon	Chondroitinase ABC	– Linear modulus, peak stress, peak strain	– Hysteresis
Legerlotz et al., 2013 [73]	Bovine extensor tendon fascicles	Chondroitinase ABC	– Failure stress, failure strain, linear modulus	↑ Stress relaxation (after 300 s)
Robinson et al., 2004 [74]	Murine tendon tail fascicles	Decorin K/O	– Peak stress, linear modulus	↓ Strain rate sensitivity
Zhang et al., 2006 [75]	Murine FDL tendon	Decorin K/O	– Peak stress, linear modulus	N/A
Dourte et al., 2012 [76]	Murine patellar tendon	Decorin K/O	– Toe modulus, linear modulus	– Phase shift
Connizzo et al., 2013 [77]	Murine SST	Decorin K/O	– Linear modulus	– Stress relaxation
		Biglycan K/O	– Linear modulus	– Stress relaxation
Dourte et al., 2013 [78]	Murine patellar tendon	Biglycan K/O	– Toe modulus, linear modulus	– Stress relaxation
			↓ Failure stress	↑ Dynamic modulus
Robinson et al., 2017 [79]	Murine patellar tendon	Decorin and biglycan K/O	– Linear modulus	↑ Stress relaxation, phase shift

(–) Indicates that there were no significant differences in the listed properties between control and treatment groups, (↑) indicates that the listed properties were significantly increased in the treatment group compared to the control group, and (↓) indicates that the listed properties were significantly decreased in the treatment group compared to the control group.

ligament's collagen structure, which is presumed to affect mechanical properties. Several studies have employed transmission electron microscopy and observed larger, more irregularly shaped collagen fibers and fibrils in decorin- or biglycan-null tendon and ligament compared to wild type tendon and ligament, which is expected given that decorin and biglycan prevent lateral fusion of adjacent fibrils [75,78,79,84,85] (we refer the interested reader to a recent publication [86] discussing tendon microscopy for further commentary on transmission electron microscopy, which has been used extensively to study tendon and ligament morphology). However, confounding variables render it impossible to determine how altered fibril diameter in the absence of decorin and biglycan contributes to the observed mechanical properties in these studies. Further, the literature continues to disagree on the role of fibril diameter in governing quasi-static mechanical properties of fibrous tissues, with varying studies supporting either a positive [65] or nonexistent [87,88] correlation between fibril diameter and elastic modulus. A novel computational or in vitro model that can independently modulate both proteoglycan–GAG bridge content and fibril diameter could advance these in vivo findings by assessing the separate impacts of proteoglycan-mediated fibril diameter changes and the proteoglycan–GAG bridge on mechanical behavior.

Another key uncertainty regarding mouse knockout models is the potential for compensation by the remaining ECM constituents. Knockout of one proteoglycan from tendon or ligament could cause upregulation of other proteoglycans that play a similar mechanical role, resulting in no discernable differences in mechanical properties between wild type and knockout tissues. Some works have highlighted that decorin-null tendons have increased biglycan expression, which could act as a functional replacement for the missing decorin [75]. Similarly, biglycan-null mouse patellar tendons were shown to have increased lumican expression but no changes in mechanical properties compared to controls [82]. These changes in ECM "background" protein content are an unavoidable consequence of mouse knockout studies that must be taken into consideration when analyzing the findings of such studies, especially if the proteins that are upregulated in the absence of a particular proteoglycan are suspected to play a role in tendon and ligament mechanics. Future mouse knockout studies should determine whether the levels of background proteins differ in wild type and knockout tissues, which will allow for a more accurate determination of whether any observed mechanical differences (or lack thereof) are caused by the genetic knockout itself or by some compensatory mechanism.

Lumican and Fibromodulin. Lumican and fibromodulin are additional members of the SLRP family that are similar to decorin and biglycan but exhibit key differences in GAG binding. Like decorin and biglycan, lumican and fibromodulin can bind to the same spot on collagen fibrils, suggesting that they have functional similarities [89]. They are also not present at the same levels in healthy animals, as fibromodulin content was shown to be six- to eightfold higher than lumican content in wild type mouse tail tendon [90]. In contrast to decorin and biglycan, fibromodulin preferentially binds to KS, while lumican is believed to exist as a glycoprotein not bound to any GAGs in musculoskeletal tissues [91]. However, to the authors' knowledge, no studies have investigated whether fibromodulin and KS form a proteoglycan–GAG bridge in tendon or ligament, or whether these proteins contribute to interfibrillar load transfer. Overall, lumican and fibromodulin are much less discussed in mechanics literature compared to decorin and biglycan despite promising evidence that they contribute to tendon and ligament mechanics.

To our knowledge, very few studies have described the mechanical contributions of lumican and fibromodulin in tendon and ligament, although those that do present strong evidence that lumican and fibromodulin are involved in regulating quasi-static fiber direction mechanical properties. A key study demonstrated

that lumican- and fibromodulin-null murine FDL tendons had a 49% reduction in fiber direction tensile modulus compared to wild type tendons [92]. Depletion of fibromodulin alone, but not lumican alone, significantly decreased stiffness and peak load despite increased levels of lumican in the fibromodulin-null tendon. However, lumican expression dictated the magnitude of stiffness reduction in fibromodulin-null tendon. Fibromodulin-null tendons with normal, heterozygous, and null lumican expression experienced 25%, 45%, and 61% reductions in stiffness, respectively, independent of cross-sectional area. These data suggest that fibromodulin has more mechanical influence than lumican, as lumican only seemed to have a noticeable mechanical contribution when fibromodulin was depleted. In addition to this work, one other study qualitatively described that cruciate ligaments from fibromodulin-null mice were more likely to exhibit visible signs of damage or rupture than cruciate ligaments from wild type mice [93]. This limited pool of data demonstrates that lumican and fibromodulin have an important yet underappreciated role in tendon and ligament mechanics, and it would be of great benefit for future studies to expand upon the knowledge described here.

Fibromodulin likely contributes to quasi-static fiber direction mechanical behavior by regulating the collagen structure during development, while lumican appears to have little, if any, contribution to this process. Svensson et al. previously showed that fibromodulin-null mouse tail tendon had thinner and less organized collagen fibrils despite a fourfold increase in lumican content [90]. Another study similarly demonstrated that fibromodulin-null and fibromodulin and lumican-null tendons had highly irregular fibril shapes and an abnormally large quantity of small fibril diameters, whereas lumican-null tendons only had minor fibril irregularities [94]. These two works implicate fibromodulin in contributing to tendon and ligament mechanics by facilitating lateral fusion of collagen fibrils, although the relationship between fibril diameter and mechanical properties remains unclear (as discussed previously). Additionally, while lumican seems to contribute little to tendon and ligament mechanics on its own, there appears to be a synergistic effect when both fibromodulin and lumican are depleted, as fibromodulin and lumican-null tendons displayed more severe decreases in mechanical properties compared to fibromodulin-null tendons alone.

Aggrecan. Aggrecan is a large proteoglycan known to govern compression mechanics in tendon and ligament. At the microscale, aggrecan is primarily located within the pericellular matrix [95]. At the macroscale, numerous works have demonstrated that aggrecan content is as much as fiftyfold higher in fibrocartilaginous regions of tendon that are mostly loaded in transverse compression compared to midsubstance regions of tendon that are mostly loaded in fiber direction tension [96–99]. It is well-known that aggrecan plays a role in compression mechanics by binding to over 100 GAGs, which attract water and increase compressive stiffness by resisting fluid flow out of the tissue [100]. While most research regarding aggrecan is focused on its ability to modulate fluid flow in articular cartilage, it is reasonable to assume that aggrecan has the same function in both fibrocartilaginous tendon and articular cartilage given their structural similarities [101,102].

Some literature has also implicated aggrecan in the tensile response of tendon and ligament, but the full details of its involvement have yet to be elucidated. Wang et al. previously showed that aggrecan accumulation in murine FDL and Achilles tendons resulted in decreased fiber direction tensile material properties (i.e., modulus, peak stress), but no changes in structural properties (i.e., stiffness, peak load) [103]. These results were attributed to increased cross-sectional area, which may have been caused by upregulation of fibromodulin or biglycan in the presence of increased aggrecan. Surprisingly, tendons with increased aggrecan content did not display changes in viscous behavior, which is unexpected given that tensile viscoelastic properties are at least partially governed by fluid expulsion [104,105]. This may have occurred because aggrecan in the tendon midsubstance has a different globular peptide structure than aggrecan in fibrocartilage and binds to few, if any, hydrophilic KS side-chains [96]. Overall, further research is needed to determine how large proteoglycans such as aggrecan interact with SLRPs to influence fiber direction tissue mechanics.

Lubricin. As its name suggests, lubricin is a proteoglycan responsible for lubricating both the interior and exterior of musculoskeletal tissues. It is present in high quantities on the exterior surfaces of tendons and ligaments, specifically in regions that are subject to shear and compression, but also exists to a lesser extent at the interfascicular and interfibrillar levels (Fig. 3(*c*)) [106,107]. Most literature discussing lubricin focuses on its ability to reduce friction between articulating joint surfaces as well as between tendon and ligament's exterior surfaces and the surrounding synovial fluid. Less is known about how lubricin contributes to the mechanics of tendon and ligament themselves, which is of most interest for the purposes of this review.

Existing studies that have measured lubricin's influence on the tendon interior have exclusively used a lubricin mouse knockout model. A key study showed that lubricin-null tendons had increased gliding resistance, defined as the force required to remove a fascicle from a tendon section by pulling in the fiber direction, which suggests that lubricin decreases interfascicular friction [108]. Another study showed that tail fascicles from lubricin-null mice had decreased fiber direction stress relaxation but no changes in Young's modulus with respect to controls [109]. Because interfibrillar sliding governs fiber direction stress relaxation (as discussed previously), this study also suggests that lubricin increases sliding at the fibril level. Similar knockout experiments are needed to confirm that lubricin also facilitates interfibrillar and interfascicular sliding during nontensile loading, which has not been directly studied despite lubricin's large presence in regions of tendon and ligament that experience shear and compression [49].

Cartilage Oligomeric Matrix Protein

Cartilage oligomeric matrix protein (COMP), also known as thrombospondin-5, is a pentameric extracellular glycoprotein located primarily within tendon and ligament fascicles [110]. Each of COMP's five subunits can bind to collagen fibrils, which has led some to suggest that COMP must modulate tendon and ligament mechanical behavior by regulating ECM assembly and structure [111]. Further, mutations in COMP are associated with development of pseudoachondroplasia, a disease that typically results in lax joints, suggesting that COMP is responsible for part of the mechanical stiffness of tendon and ligament [112].

Indeed, COMP appears to contribute to fiber direction tendon and ligament mechanics, possibly by regulating and organizing the collagen structure. A few studies have directly assessed the mechanical properties and morphology of tendon and ligament with mutated or missing COMP. One study found that murine Achilles tendons with mutated COMP had a 64% increase in failure stress and 37% increase in failure strain, but no changes in modulus, stiffness, or failure load compared to wild type tendons [113]. The tendons with mutated COMP had larger fibril diameters and decreased cross-sectional area, which likely contributed to the observed increase in failure properties. In agreement with these findings, mutated COMP has been shown to disrupt collagen fibrillogenesis in vitro [114]. In contrast, Svensson et al. found that COMP-null murine Achilles and tail tendons had no changes in fibril organization or diameter compared to wild type tendons [115]. Because of the differences observed with respect to COMP-mutated and COMP-null tendons, more work is needed to determine when and why COMP affects fibril morphology and how these changes in morphology subsequently alter tendon and ligament mechanical properties.

A few studies also indirectly indicate some involvement for COMP in fiber direction tensile mechanics, although these studies

could not successfully determine that COMP is responsible for modulating specific mechanical properties. COMP content was found to be higher in equine and bovine digital flexor tendon, an energy storing tissue, compared to digital extensor tendon, a positional tissue, indicating that COMP probably plays some role in tendon and ligament's response to tensile loads [116]. In addition, another study found positive correlations between COMP content and both ultimate tensile strength and tensile modulus in equine superficial digital flexor tendons, although this approach is flawed because other proteins that could influence mechanical properties cannot be controlled for [111]. One more recent study degraded COMP and other ECM constituents from rat tail tendon fascicles using trypsin and found no differences in quasi-static and viscoelastic mechanical behavior across several length scales, although the simultaneous depletion of other ECM constituents could mask the observation of any mechanical contribution from COMP [27]. In total, there is reason to believe that COMP plays a role in tendon and ligament mechanics, although only a few studies have directly tested this hypothesis.

Challenges and Future Directions

We have summarized the existing literature that discusses the role of several ECM components in governing mature tendon and ligament mechanical behavior. In particular, this work has expanded upon previous reviews of the non-collagenous ECM [18,117–119] by providing in-depth mechanical analysis of ECM constituents that receive less attention in tendon and ligament mechanics literature, such as lumican, fibromodulin, aggrecan, lubricin, and COMP. Our review demonstrates that the mechanical role of the non-collagenous ECM is partially defined, and that future work is still warranted in order to increase understanding within the field.

A more complete understanding of the role of the non-collagenous ECM in tendon and ligament mechanics is needed in order to define the complex relationship between ECM content, age, mechanics, and degeneration. It is well-established that tendon and ligament mechanics are altered with age [120–123] and disease [124,125]. Further, changes in non-collagenous ECM composition have been implicated in the development of tendinopathy in both young and old populations [126–129]. However, studies have typically evaluated enzyme treated or knockout tissues at one point in time, despite strong evidence that certain ECM constituents such as SLRPs play a differential role in tendon and ligament mechanics throughout development and aging [75,77,82,94,129]. Additionally, it is unclear as to whether altered ECM content contributes to disease progression, or is a phenotypic change that occurs as a result of disease progression [130]. Better characterization of the mechanical contribution of the ECM across developmental stages will further elucidate how tendon and ligament structure dictate both normal and pathological conditions.

Tissue engineering research can also be advanced by complete characterization of the role of the non-collagenous ECM in mechanics. While collagen-based scaffolds and gels are frequently the focus of functional tissue replacement efforts, constructs that incorporate natural and biomimetic elastin, decorin, and GAGs have exhibited more desirable mechanical properties compared to constructs made only of collagen [131–135]. The decellularized ECM, including both collagenous and non-collagenous components, has also been used as a scaffold for incorporation of tendon and ligament cells [135]. Therefore, further defining the contribution of the non-collagenous ECM to mechanical behavior will enable the creation of tunable tissue engineering constructs whose mechanical properties accurately replicate those of native tendon and ligament.

To address remaining knowledge gaps, several emerging areas of interest are receiving increased attention within the field. First, as mentioned in our discussion of elastin, the IFM is a new area of focus with regards to the non-collagenous matrix. In particular, the mechanical testing protocol developed by Thorpe et al. [126] has been used to identify the distinct mechanical functions of the fascicle, IFM, and whole tissue. The findings stemming from these methods implicate the IFM in tissue extension and recovery [136], fatigue resistance [31], aging [44,126], and development [137]. In addition, the IFM appears to contribute greatly to specialization of function in energy storing and positional tendons [136,138–140]. While elastin and lubricin are known to exist primarily in the IFM and contribute to interfascicular sliding [31,108], the apparent involvement of the IFM in many crucial biological and mechanical phenomena indicates that more research is warranted in order to determine how other non-collagenous constituents contribute to IFM mechanics. More specifically, while decorin, biglycan, lumican, and fibromodulin have traditionally been studied with respect to interfibrillar mechanics, evidence suggests that these SLRPs may be present within both the intra- and interfascicular matrices [139], prompting further investigation of their mechanical functions in each region.

Another area of ongoing focus is aimed at investigating the role of the ECM in fatigue damage prevention and propagation. Cyclic fatigue loading of tendon is a well-established driver of microstructural tissue damage leading to the development of tendinopathy [141–144], and recent work also indicates that microstructural fatigue damage may precede acute ligament injuries [6]. While elastin is widely known for its fatigue resistance properties [31,43], the role of other non-collagenous ECM constituents in fatigue mechanics is unclear. One study suggested that GAGs provide fatigue resistance by limiting viscoelastic behavior, although this hypothesis was not directly evaluated [73]. To the authors' knowledge, no other studies have commented on this topic. Cyclic fatigue loading of tissues deficient in proteoglycans, GAGs, or other ECM constituents will advance our knowledge in this area. This work will prove crucial in determining the role of the non-collagenous ECM in tendon and ligament mechanical behavior, as well as highlight the important role of these proteins in homeostasis and disease.

Finally, the lack of evidence clearly supporting a role for the proteoglycan–GAG bridge in interfascicular load transfer has led to new theories to explain previous findings in this area. A key hypothesis suggests that minor collagen constituents facilitate load transfer and fibril sliding in tendon and ligament. One study did not observe differences in mechanical properties when most of the non-collagenous ECM was degraded using trypsin, but did observe small collagen fibrils forming bridges between larger collagen fibrils, suggesting that interfibrillar load transfer may actually be facilitated by collagen itself (Fig. 5) [27]. Collagen branching in developing and mature fibrils and fibers has also been reported elsewhere [145–147], which lends support to this theory and could explain why some studies have observed collagen fibers that appear continuous throughout the length of the tissue [146,148]. The mechanical contribution of branching fibrils can be further assessed by incorporating depletion of branching fibrils into existing computational models of fibril mechanics [149], or by characterizing the composition of branching fibrils, which is currently unknown [27], and developing a depletion or knockout model to allow for experimental analysis of tissues lacking branching fibrils.

The largest barriers that prevent a full understanding of the relationship between the tendon and ligament ECM and mechanical properties are the lack of breadth of enzyme degradation studies and the limitations of other study methods. While mouse knockout studies offer the ability to remove almost any component from the ECM, they may be limited by compensation from background proteins, as was discussed previously. Inducible mouse knockout models have made progress in this area by allowing for depletion of proteins of interest in mature animals, thus eliminating compensation effects during development [79]. However, some knockout studies can only compare haploinsufficient and wild type animals because a full knockout creates nonviable animals, such as in the case of elastin [54]. This limits the amount of the

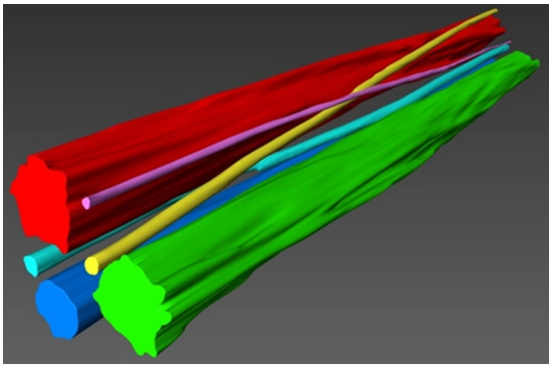

Fig. 5 Three-dimensional reconstruction of collagen fibrils in rat tail tendon fascicles. Images were obtained using serial block-face scanning electron microscopy to capture cross section throughout the fibril length. Small diameter fibrils were observed to link adjacent to large diameter fibrils. This finding could explain why many studies have observed interfibrillar sliding and load transfer even though the non-collagenous proteoglycan–GAG bridge may not be implicated in these processes. Reprinted with permission from Szczesny et al. [27]. Copyright 2017 by Wiley.

component of interest that can be removed from the tissue and prevents studies from describing the full spectrum of mechanical changes that occur when the component of interest is mostly or fully removed. It is also important to acknowledge that differences in tendon and ligament structure between small animals and humans may limit the applicability of results from small animal models; we refer the reader to an interesting publication by Lee and Elliott for further discussion of this topic [19]. In addition, indirect methods of assessing a protein's mechanical role, such as measuring protein content in regions of tendon or ligament that experience different types of loading, do not provide any evidence that the protein of interest directly contributes to observed mechanical properties. Because of these limitations, in vitro enzyme degradation studies remain the most viable method of assessing an ECM constituent's contribution to mechanical properties, although these have only been performed with elastase and chondroitinase to date. The advantages and disadvantages of techniques used to model tendon and ligament function have also been reviewed elsewhere, with the authors concluding that tissue explant models optimally mimic the native tissue structure without confounding biological complexity in comparison to in vitro cell culture and in vivo models [150].

As evidenced, there is a paramount need to identify enzymes that can degrade ECM components not previously investigated via enzyme degradation in vitro. Several members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzyme family have been shown to degrade COMP, aggrecan, and versican in tissues other than tendon and ligament, which could be a useful starting point for future studies [151]. Further investigation of the ability of these enzymes to degrade ECM constituents from tendon and ligament with high specificity has the potential to greatly enhance the methods available for the assessment of tendon and ligament structure–function relationships.

In conclusion, the role of the non-collagenous ECM in tendon and ligament mechanics is partially understood. While the mechanical roles of elastin, lumican, fibromodulin, aggrecan, and lubricin are at least somewhat clear, other constituents such as decorin, biglycan, and COMP have been implicated in mechanics with less understanding of actual structure–function relationships. The roles of decorin, biglycan, and sulfated GAGs remain particularly controversial, as various study methodologies have produced conflicting results regarding the ability of the proteoglycan–GAG bridge to transfer loads between adjacent collagen fibrils. Additionally, while quasi-static mechanical tests are frequently used to define structure–function relationships, more research is needed to determine which non-collagenous ECM constituents provide tendon and ligament with their characteristic viscoelastic responses. The studies presented here and those that will be conducted in the future will foster a better understanding of homeostatic and pathologic tendon and ligament function, increase knowledge of tendon and ligament development and aging, and improve tissue regeneration and replacement strategies.

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Nomenclature

ACL = anterior cruciate ligament
COMP = cartilage oligomeric matrix protein
CS = chondroitin sulfate
DS = dermatan sulfate
ECM = extracellular matrix
FDL = flexor digitorum longus
GAG = glycosaminoglycan
IFM = interfascicular matrix
K/O = knockout
KS = keratan sulfate
MCL = medial collateral ligament
SLRP = small leucine-rich proteoglycan
SST = supraspinatus tendon

References

[1] Renstrom, P. A. H. F., and Woo, S. L.-Y., 2007, "Tendinopathy: A Major Medical Problem in Sport," *Tendinopathy in Athletes*, Wiley Blackwell, Hoboken, NJ, pp. 1–9.
[2] Riel, H., Lindstrøm, C. F., Rathleff, M. S., Jensen, M. B., and Olesen, J. L., 2019, "Prevalence and Incidence Rate of Lower-Extremity Tendinopathies in a Danish General Practice: A Registry-Based Study," BMC Musculoskeletal Disord., **20**(1), p. 239.
[3] Sanders, T. L., Maradit Kremers, H., Bryan, A. J., Larson, D. R., Dahm, D. L., Levy, B. A., Stuart, M. J., and Krych, A. J., 2016, "Incidence of Anterior Cruciate Ligament Tears and Reconstruction: A 21-Year Population-Based Study," Am. J. Sports Med., **44**(6), pp. 1502–1507.
[4] Dan, M., Parr, W., Broe, D., Cross, M., and Walsh, W. R., 2018, "Biomechanics of the Knee Extensor Mechanism and Its Relationship to Patella Tendinopathy: A Review," J. Orthop. Res., **36**(12), pp. 3105–3112.
[5] Nakama, L. H., King, K. B., Abrahamsson, S., and Rempel, D. M., 2005, "Evidence of Tendon Microtears Due to Cyclical Loading in an In Vivo Tendinopathy Model," J. Orthop. Res., **23**(5), pp. 1199–1205.
[6] Chen, J., Kim, J., Shao, W., Schlecht, S. H., Baek, S. Y., Jones, A. K., Ahn, T., Ashton-Miller, J. A., Banaszak Holl, M. M., and Wojtys, E. M., 2019, "An Anterior Cruciate Ligament Failure Mechanism," Am. J. Sports Med., **47**(9), pp. 2067–2076.
[7] Quapp, K. M., and Weiss, J. A., 1998, "Material Characterization of Human Medial Collateral Ligament," ASME J. Biomech. Eng., **120**(6), pp. 757–763.
[8] Chandrashekar, N., Hashemi, J., Slauterbeck, J., and Beynnon, B. D., 2008, "Low-Load Behaviour of the Patellar Tendon Graft and Its Relevance to the Biomechanics of the Reconstructed Knee," Clin. Biomech., **23**(7), pp. 918–925.
[9] Miller, K. S., Connizzo, B. K., Feeney, E., and Soslowsky, L. J., 2012, "Characterizing Local Collagen Fiber Re-Alignment and Crimp Behavior Throughout Mechanical Testing in a Mature Mouse Supraspinatus Tendon Model," J. Biomech., **45**(12), pp. 2061–2065.
[10] Cheng, V. W. T., and Screen, H. R. C., 2007, "The Micro-Structural Strain Response of Tendon," J. Mater. Sci., **42**(21), pp. 8957–8965.
[11] Rumian, A. P., Wallace, A. L., and Birch, H. L., 2007, "Tendons and Ligaments Are Anatomically Distinct but Overlap in Molecular and Morphological Features—A Comparative Study in an Ovine Model," J. Orthop. Res., **25**(4), pp. 458–464.
[12] Screen, H. R. C., 2009, "Hierarchical Approaches to Understanding Tendon Mechanics," J. Biomech. Sci. Eng., **4**(4), pp. 481–499.
[13] Peterson, B. E., and Szczesny, S. E., 2020, "Dependence of Tendon Multiscale Mechanics on Sample Gauge Length Is Consistent With Discontinuous Collagen Fibrils," Acta Biomater., **117**, pp. 302–309.

[14] Fratzl, P., 2008, *Collagen: Structure and Mechanics*, Springer Science and Business Media LLC, New York.
[15] Kastelic, J., Galeski, A., and Baer, E., 1978, "The Multicomposite Structure of Tendon," Connect. Tissue Res., **6**(1), pp. 11–23.
[16] Amiel, D., Frank, C., Harwood, F., Fronek, J., and Akeson, W., 1983, "Tendons and Ligaments: A Morphological and Biochemical Comparison," J. Orthop. Res., **1**(3), pp. 257–265.
[17] Koob, T. J., and Vogel, K. G., 1987, "Site-Related Variations in Glycosaminoglycan Content and Swelling Properties of Bovine Flexor Tendon," J. Orthop. Res., **5**(3), pp. 414–424.
[18] Fang, F., and Lake, S. P., 2017, "Experimental Evaluation of Multiscale Tendon Mechanics," J. Orthop. Res., **35**(7), pp. 1353–1365.
[19] Lee, A. H., and Elliott, D. M., 2019, "Comparative Multi-Scale Hierarchical Structure of the Tail, Plantaris, and Achilles Tendons in the Rat," J. Anat., **234**(2), pp. 252–262.
[20] Szczesny, S. E., and Elliott, D. M., 2014, "Interfibrillar Shear Stress Is the Loading Mechanism of Collagen Fibrils in Tendon," Acta Biomater., **10**(6), pp. 2582–2590.
[21] Obuchowicz, R., Ekiert, M., Kohut, P., Holak, K., Ambrozinski, L., Tomaszewski, K. A., Uhl, T., and Mlyniec, A., 2019, "Interfascicular Matrix-Mediated Transverse Deformation and Sliding of Discontinuous Tendon Subcomponents Control the Viscoelasticity and Failure of Tendons," J. Mech. Behav. Biomed. Mater., **97**, pp. 238–246.
[22] Screen, H. R. C., 2008, "Investigating Load Relaxation Mechanics in Tendon," J. Mech. Behav. Biomed. Mater., **1**(1), pp. 51–58.
[23] Gupta, H. S., Seto, J., Krauss, S., Boesecke, P., and Screen, H. R. C., 2010, "In Situ Multi-Level Analysis of Viscoelastic Deformation Mechanisms in Tendon Collagen," J. Struct. Biol., **169**(2), pp. 183–191.
[24] Ahmadzadeh, H., Connizzo, B. K., Freedman, B. R., Soslowsky, L. J., and Shenoy, V. B., 2013, "Determining the Contribution of Glycosaminoglycans to Tendon Mechanical Properties With a Modified Shear-Lag Model," J. Biomech., **46**(14), pp. 2497–2503.
[25] Fessel, G., and Snedeker, J. G., 2009, "Evidence Against Proteoglycan Mediated Collagen Fibril Load Transmission and Dynamic Viscoelasticity in Tendon," Matrix Biol., **28**(8), pp. 503–510.
[26] Svensson, R. B., Hassenkam, T., Hansen, P., Kjaer, M., and Magnusson, S. P., 2011, "Tensile Force Transmission in Human Patellar Tendon Fascicles Is Not Mediated by Glycosaminoglycans," Connect. Tissue Res., **52**(5), pp. 415–421.
[27] Szczesny, S. E., Fetchko, K. L., Dodge, G. R., and Elliott, D. M., 2017, "Evidence That Interfibrillar Load Transfer in Tendon Is Supported by Small Diameter Fibrils and Not Extrafibrillar Tissue Components," J. Orthop. Res., **35**(10), pp. 2127–2134.
[28] Fang, F., and Lake, S. P., 2015, "Multiscale Strain Analysis of Tendon Subjected to Shear and Compression Demonstrates Strain Attenuation, Fiber Sliding, and Reorganization," J. Orthop. Res., **33**(11), pp. 1704–1712.
[29] Fang, F., and Lake, S. P., 2017, "Multiscale Mechanical Evaluation of Human Supraspinatus Tendon Under Shear Loading After Glycosaminoglycan Reduction," ASME J. Biomech. Eng., **139**(7), p. 071013.
[30] Henninger, H. B., Valdez, W. R., Scott, S. A., and Weiss, J. A., 2015, "Elastin Governs the Mechanical Response of Medial Collateral Ligament Under Shear and Transverse Tensile Loading," Acta Biomater., **25**, pp. 304–312.
[31] Godinho, M. S., Thorpe, C. T., Greenwald, S. E., and Screen, H. R. C., 2021, "Elastase Treatment of Tendon Specifically Impacts the Mechanical Properties of the Interfascicular Matrix," Acta Biomater., **123**, pp. 187–196.
[32] Han, W. M., Heo, S. J., Driscoll, T. P., Smith, L. J., Mauck, R. L., and Elliott, D. M., 2013, "Macro- to Microscale Strain Transfer in Fibrous Tissues Is Heterogeneous and Tissue-Specific," Biophys. J., **105**(3), pp. 807–817.
[33] Arnoczky, S. P., Lavagnino, M., Whallon, J. H., and Hoonjan, A., 2002, "In Situ Cell Nucleus Deformation in Tendons Under Tensile Load; a Morphological Analysis Using Confocal Laser Microscopy," J. Orthop. Res., **20**(1), pp. 29–35.
[34] Lavagnino, M., and Arnoczky, S. P., 2005, "In Vitro Alterations in Cytoskeletal Tensional Homeostasis Control Gene Expression in Tendon Cells," J. Orthop. Res., **23**(5), pp. 1211–1218.
[35] Ritty, T. M., Roth, R., and Heuser, J. E., 2003, "Tendon Cell Array Isolation Reveals a Previously Unknown Fibrillin-2-Containing Macromolecular Assembly," Structure, **11**(9), pp. 1179–1188.
[36] Hae Yoon, J., Brooks, R., Hwan Kim, Y., Terada, M., and Halper, J., 2003, "Proteoglycans in Chicken Gastrocnemius Tendons Change With Exercise," Arch. Biochem. Biophys., **412**(2), pp. 279–286.
[37] Franchi, M., Torricelli, P., Giavaresi, G., and Fini, M., 2013, "Role of Moderate Exercising on Achilles Tendon Collagen Crimping Patterns and Proteoglycans," Connect. Tissue Res., **54**(4–5), pp. 267–274.
[38] Frizziero, A., Fini, M., Salamanna, F., Veicsteinas, A., Maffulli, N., and Martini, M., 2011, "Effect of Training and Sudden Detraining on the Patellar Tendon and Its Enthesis in Rats," BMC Musculoskeletal Disord., **12**(1), p. 20.
[39] Lavagnino, M., Wall, M. E., Little, D., Banes, A. J., Guilak, F., and Arnoczky, S. P., 2015, "Tendon Mechanobiology: Current Knowledge and Future Research Opportunities," J. Orthop. Res., **33**(6), pp. 813–822.
[40] Rees, S. G., Dent, C. M., and Caterson, B., 2009, "Metabolism of Proteoglycans in Tendon," Scand. J. Med. Sci. Sports, **19**(4), pp. 470–478.
[41] Chatterjee, M., Muljadi, P. M., and Andarawis-Puri, N., 2021, "The Role of the Tendon ECM in Mechanotransduction Disruption and Repair Following Overuse," Connect. Tissue Res., pp. 1–15.
[42] Wall, M., Butler, D., el Haj, A., Bodle, J. C., Loboa, E. G., and Banes, A. J., 2017, "Key Developments That Impacted the Field of Mechanobiology and Mechanotransduction," J. Orthop. Res., **36**(2), pp. 605–619.
[43] Gosline, J., Lillie, M., Carrington, E., Guerette, P., Ortlepp, C., and Savage, K., 2002, "Elastic Proteins: Biological Roles and Mechanical Properties," Philos. Trans. R. Soc. B: Biol. Sci., **357**(1418), pp. 121–132.
[44] Godinho, M. S. C., Thorpe, C. T., Greenwald, S. E., and Screen, H. R. C., 2017, "Elastin Is Localised to the Interfascicular Matrix of Energy Storing Tendons and Becomes Increasingly Disorganised With Ageing," Sci. Rep., **7**(1), p. 9713.
[45] Kielty, C. M., Sherratt, M. J., and Shuttleworth, C. A., 2002, "Elastic Fibres," J. Cell Sci., **115**(14), pp. 2817–2828.
[46] Lorber, M., 1989, "Elastic Fibers in the Rat Exorbital Lacrimal Gland Duct System," Invest. Ophthalmol. Visual Sci., **30**(9), pp. 2002–2011.
[47] Grant, T. M., Thompson, M. S., Urban, J., and Yu, J., 2013, "Elastic Fibres Are Broadly Distributed in Tendon and Highly Localized Around Tenocytes," J. Anat., **222**(6), pp. 573–579.
[48] Liao, J., and Vesely, I., 2007, "Skewness Angle of Interfibrillar Proteoglycans Increases With Applied Load on Mitral Valve Chordae Tendineae," J. Biomech., **40**(2), pp. 390–398.
[49] Sun, Y. L., Wei, Z., Zhao, C., Jay, G. D., Schmid, T. M., Amadio, P. C., and An, K. N., 2015, "Lubricin in Human Achilles Tendon: The Evidence of Intratendinous Sliding Motion and Shear Force in Achilles Tendon," J. Orthop. Res., **33**(6), pp. 932–937.
[50] Henninger, H. B., Underwood, C. J., Romney, S. J., Davis, G. L., and Weiss, J. A., 2013, "Effect of Elastin Digestion on the Quasi-Static Tensile Response of Medial Collateral Ligament," J. Orthop. Res., **31**(8), pp. 1226–1233.
[51] Reihsner, R., Menzel, E. J., Mallinger, R., and Millesi, H., 1991, "Biomechanical Properties of Elastase Treated Palmar Aponeuroses," Connect. Tissue Res., **26**(1–2), pp. 77–86.
[52] Grant, T. M., Yapp, C., Chen, Q., Czernuszka, J. T., and Thompson, M. S., 2015, "The Mechanical, Structural, and Compositional Changes of Tendon Exposed to Elastase," Ann. Biomed. Eng., **43**(10), pp. 2477–2486.
[53] Palmer, J. S., and Boyce, M. C., 2008, "Constitutive Modeling of the Stress-Strain Behavior of F-Actin Filament Networks," Acta Biomater., **4**(3), pp. 597–612.
[54] Eekhoff, J. D., Fang, F., Kahan, L. G., Espinosa, G., Cocciolone, A. J., Wagenseil, J. E., Mecham, R. P., and Lake, S. P., 2017, "Functionally Distinct Tendons From Elastin Haploinsufficient Mice Exhibit Mild Stiffening and Tendon-Specific Structural Alteration," ASME J. Biomech. Eng., **139**(11), p. 111003.
[55] Millesi, H., Reihsner, R., Hamilton, G., Mallinger, R., and Menzel, E. J., 1995, "Biomechanical Properties of Normal Tendons, Normal Palmar Aponeuroses and Palmar Aponeuroses From Patients With Dupuytren's Disease Subjected to Elastase and Chondroitinase Treatment," Connect. Tissue Res., **31**(2), pp. 109–115.
[56] Fang, F., and Lake, S. P., 2016, "Multiscale Mechanical Integrity of Human Supraspinatus Tendon in Shear After Elastin Depletion," J. Mech. Behav. Biomed. Mater., **63**, pp. 443–455.
[57] Samiric, T., Ilic, M. Z., and Handley, C. J., 2004, "Characterisation of Proteoglycans and Their Catabolic Products in Tendon and Explant Cultures of Tendon," Matrix Biol., **23**(2), pp. 127–140.
[58] Yoon, J. H., and Halper, J., 2005, "Tendon Proteoglycans: Biochemistry and Function," J. Musculoskeletal Neuronal Interact., **5**(1), pp. 22–34.
[59] Chen, S., and Birk, D. E., 2013, "The Regulatory Roles of Small Leucine-Rich Proteoglycans in Extracellular Matrix Assembly," FEBS J., **280**(10), pp. 2120–2137.
[60] Connizzo, B. K., Yannascoli, S. M., and Soslowsky, L. J., 2013, "Structure-Function Relationships of Postnatal Tendon Development: A Parallel to Healing," Matrix Biol., **32**(2), pp. 106–116.
[61] Henninger, H. B., Underwood, C. J., Ateshian, G. A., and Weiss, J. A., 2010, "Effect of Sulfated Glycosaminoglycan Digestion on the Transverse Permeability of Medial Collateral Ligament," J. Biomech., **43**(13), pp. 2567–2573.
[62] Cribb, A. M., and Scott, J. E., 1995, "Tendon Response to Tensile Stress: An Ultrastructural Investigation of Collagen:Proteoglycan Interactions in Stressed Tendon," J. Anat., **187**, pp. 423–428.
[63] Scott, J. E., 2001, "Structure and Function in Extracellular Matrices Depend on Interactions Between Anionic Glycosaminoglycans," Pathol. Biol., **49**(4), pp. 284–293.
[64] Vesentini, S., Redaelli, A., and Montevecchi, F. M., 2005, "Estimation of the Binding Force of the Collagen Molecule-Decorin Core Protein Complex in Collagen Fibril," J. Biomech., **38**(3), pp. 433–443.
[65] Redaelli, A., Vesentini, S., Soncini, M., Vena, P., Mantero, S., and Montevecchi, F. M., 2003, "Possible Role of Decorin Glycosaminoglycans in Fibril to Fibril Force Transfer in Relative Mature Tendons—A Computational Study From Molecular to Microstructural Level," J. Biomech., **36**(10), pp. 1555–1569.
[66] Ahmadzadeh, H., Freedman, B. R., Connizzo, B. K., Soslowsky, L. J., and Shenoy, V. B., 2015, "Micromechanical Poroelastic Finite Element and Shear-Lag Models of Tendon Predict Large Strain Dependent Poisson's Ratios and Fluid Expulsion Under Tensile Loading," Acta Biomater., **22**, pp. 83–91.
[67] Ciarletta, P., Dario, P., and Micera, S., 2008, "Pseudo-Hyperelastic Model of Tendon Hysteresis From Adaptive Recruitment of Collagen Type I Fibrils," Biomaterials, **29**(6), pp. 764–770.
[68] Fessel, G., and Snedeker, J. G., 2011, "Equivalent Stiffness After Glycosaminoglycan Depletion in Tendon—An Ultra-Structural Finite Element Model and Corresponding Experiments," J. Theor. Biol., **268**(1), pp. 77–83.
[69] Liu, X., Yeh, M. L., Lewis, J. L., and Luo, Z. P., 2005, "Direct Measurement of the Rupture Force of Single Pair of Decorin Interactions," Biochem. Biophys. Res. Commun., **338**(3), pp. 1342–1345.

[70] Lujan, T. J., Underwood, C. J., Henninger, H. B., Thompson, B. M., and Weiss, J. A., 2007, "Effect of Dermatan Sulfate Glycosaminoglycans on the Quasi-Static Material Properties of the Human Medial Collateral Ligament," J. Orthop. Res., **25**(7), pp. 894–903.
[71] Lujan, T. J., Underwood, C. J., Jacobs, N. T., and Weiss, J. A., 2009, "Contribution of Glycosaminoglycans to Viscoelastic Tensile Behavior of Human Ligament," J. Appl. Physiol., **106**(2), pp. 423–431.
[72] Rigozzi, S., Müller, R., and Snedeker, J. G., 2009, "Local Strain Measurement Reveals a Varied Regional Dependence of Tensile Tendon Mechanics on Glycosaminoglycan Content," J. Biomech., **42**(10), pp. 1547–1552.
[73] Legerlotz, K., Riley, G. P., and Screen, H. R. C., 2013, "GAG Depletion Increases the Stress-Relaxation Response of Tendon Fascicles, but Does Not Influence Recovery," Acta Biomater., **9**(6), pp. 6860–6866.
[74] Robinson, P. S., Lin, T. W., Reynolds, P. R., Derwin, K. A., Iozzo, R. V., and Soslowsky, L. J., 2004, "Strain-Rate Sensitive Mechanical Properties of Tendon Fascicles From Mice With Genetically Engineered Alterations in Collagen and Decorin," ASME J. Biomech. Eng., **126**(2), pp. 252–257.
[75] Zhang, G., Ezura, Y., Chervoneva, I., Robinson, P. S., Beason, D. P., Carine, E. T., Soslowsky, L. J., Iozzo, R. V., and Birk, D. E., 2006, "Decorin Regulates Assembly of Collagen Fibrils and Acquisition of Biomechanical Properties During Tendon Development," J. Cell. Biochem., **98**(6), pp. 1436–1449.
[76] Dourte, L. M., Pathmanathan, L., Jawad, A. F., Iozzo, R. V., Mienaltowski, M. J., Birk, D. E., and Soslowsky, L. J., 2012, "Influence of Decorin on the Mechanical, Compositional, and Structural Properties of the Mouse Patellar Tendon," ASME J. Biomech. Eng., **134**(3), p. 031005.
[77] Connizzo, B. K., Sarver, J. J., Iozzo, R. V., Birk, D. E., and Soslowsky, L. J., 2013, "Effect of Age and Proteoglycan Deficiency on Collagen Fiber Re-Alignment and Mechanical Properties in Mouse Supraspinatus Tendon," ASME J. Biomech. Eng., **135**(2), p. 021019.
[78] Dourte, L. M., Pathmanathan, L., Mienaltowski, M. J., Jawad, A. F., Birk, D. E., and Soslowsky, L. J., 2013, "Mechanical, Compositional, and Structural Properties of the Mouse Patellar Tendon With Changes in Biglycan Gene Expression," J. Orthop. Res., **31**(9), pp. 1430–1437.
[79] Robinson, K. A., Sun, M., Barnum, C. E., Weiss, S. N., Huegel, J., Shetye, S. S., Lin, L., 2017, "Decorin and Biglycan Are Necessary for Maintaining Collagen Fibril Structure, Fiber Realignment, and Mechanical Properties of Mature Tendons," Matrix Biol., **64**, pp. 81–93.
[80] Fung, Y. C., 1981, *Biomechanics: Mechanical Properties of Living Tissues*, Springer New York, New York.
[81] Robinson, P. S., Huang, T. F., Kazam, E., Iozzo, R. V., Birk, D. E., and Soslowsky, L. J., 2005, "Influence of Decorin and Biglycan on Mechanical Properties of Multiple Tendons in Knockout Mice," ASME J. Biomech. Eng., **127**(1), pp. 181–185.
[82] Dunkman, A. A., Buckley, M. R., Mienaltowski, M. J., Adams, S. M., Thomas, S. J., Satchell, L., Kumar, A., 2013, "Decorin Expression Is Important for Age-Related Changes in Tendon Structure and Mechanical Properties," Matrix Biol., **32**(1), pp. 3–13.
[83] Corsi, A., Xu, T., Chen, X.-D., Boyde, A., Liang, J., Mankani, M., Sommer, B., 2002, "Phenotypic Effects of Biglycan Deficiency Are Linked to Collagen Fibril Abnormalities, Are Synergized by Decorin Deficiency, and Mimic Ehlers-Danlos-Like Changes in Bone and Other Connective Tissues," J. Bone Miner. Res., **17**(7), pp. 1180–1189.
[84] Danielson, K. G., Baribault, H., Holmes, D. F., Graham, H., Kadler, K. E., and Iozzo, R. V., 1997, "Targeted Disruption of Decorin Leads to Abnormal Collagen Fibril Morphology and Skin Fragility," J. Cell Biol., **136**(3), pp. 729–743.
[85] Häkkinen, L., Strassburger, S., Kähäri, V.-M., Scott, P. G., Eichstetter, I., Iozzo, R. V., and Larjava, H., 2000, "A Role for Decorin in the Structural Organization of Periodontal Ligament," Lab. Invest., **80**(12), pp. 1869–1880.
[86] Xu, M., Liu, J., Sun, J., Xu, X., Hu, Y., and Liu, B., 2020, "Optical Microscopy and Electron Microscopy for the Morphological Evaluation of Tendons: A Mini Review," Orthop. Surg., **12**(2), pp. 366–371.
[87] Christiansen, D. L., Huang, E. K., and Silver, F. H., 2000, "Assembly of Type I Collagen: Fusion of Fibril Subunits and the Influence of Fibril Diameter on Mechanical Properties," Matrix Biol., **19**(5), pp. 409–420.
[88] Wenger, M. P. E., Bozec, L., Horton, M. A., and Mesquida, P., 2007, "Mechanical Properties of Collagen Fibrils," Biophys. J., **93**(4), pp. 1255–1263.
[89] Chakravarti, S., 2002, "Functions of Lumican and Fibromodulin: Lessons From Knockout Mice," Glycoconjugate J., **19**(4/5), pp. 287–293.
[90] Svensson, L., Aszódi, A., Reinholt, F. P., Fässler, R., Heinegård, D., and Oldberg, Å., 1999, "Fibromodulin-Null Mice Have Abnormal Collagen Fibrils, Tissue Organization, and Altered Lumican Deposition in Tendon," J. Biol. Chem., **274**(14), pp. 9636–9647.
[91] Ying, S., Shiraishi, A., Kao, C. W. C., Converse, R. L., Funderburgh, J. L., Swiergiel, J., Roth, M. R., Conrad, G. W., and Kao, W. W. Y., 1997, "Characterization and Expression of the Mouse Lumican Gene," J. Biol. Chem., **272**(48), pp. 30306–30313.
[92] Jepsen, K. J., Wu, F., Peragallo, J. H., Paul, J., Roberts, L., Ezura, Y., Oldberg, A., Birk, D. E., and Chakravarti, S., 2002, "A Syndrome of Joint Laxity and Impaired Tendon Integrity in Lumican- and Fibromodulin-Deficient Mice," J. Biol. Chem., **277**(38), pp. 35532–35540.
[93] Gill, M. R., Oldberg, Å., and Reinholt, F. P., 2002, "Fibromodulin-Null Murine Knee Joints Display Increased Incidences of Osteoarthritis and Alterations in Tissue Biochemistry," Osteoarthritis Cartilage, **10**(10), pp. 751–757.
[94] Ezura, Y., Chakravarti, S., Oldberg, Å., Chervoneva, I., and Birk, D. E., 2000, "Differential Expression of Lumican and Fibromodulin Regulate Collagen Fibrillogenesis in Developing Mouse Tendons," J. Cell Biol., **151**(4), pp. 779–787.
[95] Bell, R., Li, J., Gorski, D. J., Bartels, A. K., Shewman, E. F., Wysocki, R. W., Cole, B. J., 2013, "Controlled Treadmill Exercise Eliminates Chondroid Deposits and Restores Tensile Properties in a New Murine Tendinopathy Model," J. Biomech., **46**(3), pp. 498–505.
[96] Vogel, K. G., Sandy, J. D., Pogany, G., and Robbins, J. R., 1994, "Aggrecan in Bovine Tendon," Matrix Biol., **14**(2), pp. 171–179.
[97] Martin, J. A., Mehr, D., Pardubsky, P. D., and Buckwalter, J. A., 2003, "The Role of Tenascin-C in Adaptation of Tendons to Compressive Loading," Biorheology, **40**(1–3), pp. 321–329.
[98] Matuszewski, P. E., Chen, Y. L., Szczesny, S. E., Lake, S. P., Elliott, D. M., Soslowsky, L. J., and Dodge, G. R., 2012, "Regional Variation in Human Supraspinatus Tendon Proteoglycans: Decorin, Biglycan, and Aggrecan," Connect. Tissue Res., **53**(5), pp. 343–348.
[99] Huisman, E. S., Andersson, G., Scott, A., Reno, C. R., Hart, D. A., and Thornton, G. M., 2014, "Regional Molecular and Cellular Differences in the Female Rabbit Achilles Tendon Complex: Potential Implications for Understanding Responses to Loading," J. Anat., **224**(5), pp. 538–547.
[100] Rees, S. G., Waggett, A. D., Dent, C. M., and Caterson, B., 2007, "Inhibition of Aggrecan Turnover in Short-Term Explant Cultures of Bovine Tendon," Matrix Biol., **26**(4), pp. 280–290.
[101] Benjamin, M., and Ralphs, J. R., 1998, "Fibrocartilage in Tendons and Ligaments—An Adaptation to Compressive Load," J. Anat., **193**(4), pp. 481–494.
[102] Vogel, K., 2004, "What Happens When Tendons Bend and Twist? Proteoglycans," J. Musculoskeletal Neuronal Interact., **4**(2), pp. 202–203.
[103] Wang, V. M., Bell, R. M., Thakore, R., Eyre, D. R., Galante, J. O., Li, J., Sandy, J. D., and Plaas, A., 2012, "Murine Tendon Function Is Adversely Affected by Aggrecan Accumulation Due to the Knockout of ADAMTS5," J. Orthop. Res., **30**(4), pp. 620–626.
[104] Yin, L., and Elliott, D. M., 2004, "A Biphasic and Transversely Isotropic Mechanical Model for Tendon: Application to Mouse Tail Fascicles in Uniaxial Tension," J. Biomech., **37**(6), pp. 907–916.
[105] Reese, S. P., and Weiss, J. A., 2013, "Tendon Fascicles Exhibit a Linear Correlation Between Poisson's Ratio and Force During Uniaxial Stress Relaxation," ASME J. Biomech. Eng., **135**(3), p. 034501.
[106] Sun, Y., Berger, E. J., Zhao, C., Jay, G. D., An, K. N., and Amadio, P. C., 2006, "Expression and Mapping of Lubricin in Canine Flexor Tendon," J. Orthop. Res., **24**(9), pp. 1861–1868.
[107] Funakoshi, T., Schmid, T., Hsu, H. P., and Spector, M., 2008, "Lubricin Distribution in the Goat Infraspinatus Tendon: A Basis for Interfascicular Lubrication," J. Bone Jt. Surg. - Ser. A, **90**(4), pp. 803–814.
[108] Kohrs, R. T., Zhao, C., Sun, Y. L., Jay, G. D., Zhang, L., Warman, M. L., An, K. N., and Amadio, P. C., 2011, "Tendon Fascicle Gliding in Wild Type, Heterozygous, and Lubricin Knockout Mice," J. Orthop. Res., **29**(3), pp. 384–389.
[109] Reuvers, J., Thoreson, A. R., Zhao, C., Zhang, L., Jay, G. D., An, K. N., Warman, M. L., and Amadio, P. C., 2011, "The Mechanical Properties of Tail Tendon Fascicles From Lubricin Knockout, Wild Type and Heterozygous Mice," J. Struct. Biol., **176**(1), pp. 41–45.
[110] Södersten, F., Ekman, S., Eloranta, M. L., Heinegård, D., Dudhia, J., and Hultenby, K., 2005, "Ultrastructural Immunolocalization of Cartilage Oligomeric Matrix Protein (COMP) in Relation to Collagen Fibrils in the Equine Tendon," Matrix Biol., **24**(5), pp. 376–385.
[111] Smith, R., Gerard, M., Dowling, B., Dart, A., Birch, H., and Goodship, E., 2010, "Correlation of Cartilage Oligomeric Matrix Protein (COMP) Levels in Equine Tendon With Mechanical Properties: A Proposed Role for COMP in Determining Function-Specific Mechanical Characteristics of Locomotor Tendons," Equine Vet. J., **34**(S34), pp. 241–244.
[112] Briggs, M., Hoffman, S., King, L., Olsen, A., Mohrenweiser, H., Leroy, J., Mortier, G., 1995, "Pseudoachondroplasia and Multiple Epiphyseal Dysplasia Due to Mutations in the Cartilage Oligomeric Matrix Protein Gene," Nat. Genet., **10**(3), pp. 330–336.
[113] Piróg, K. A., Jaka, O., Katakura, Y., Meadows, R. S., Kadler, K. E., Boot-Handford, R. P., and Briggs, M. D., 2010, "A Mouse Model Offers Novel Insights Into the Myopathy and Tendinopathy Often Associated With Pseudoachondroplasia and Multiple Epiphyseal Dysplasia," Hum. Mol. Genet., **19**(1), pp. 52–64.
[114] Hansen, U., Platz, N., Becker, A., Bruckner, P., Paulsson, M., and Zaucke, F., 2011, "A Secreted Variant of Cartilage Oligomeric Matrix Protein Carrying a Chondrodysplasia-Causing Mutation (p.H587R) Disrupts Collagen Fibrillogenesis," Arthritis Rheum., **63**(1), pp. 159–167.
[115] Svensson, L., Aszódi, A., Heinega˚rd, D., Hunziker, E. B., Reinholt, F. P., Fässler, R., and Oldberg, Å., 2002, "Cartilage Oligomeric Matrix Protein-Deficient Mice Have Normal Skeletal Development," Mol. Cell. Biol., **22**(12), pp. 4366–4371.
[116] Smith, R., Zunino, L., Webbon, P., and Heinegard, D., 1997, "The Distribution of Cartitage Oligomeric Matrix Protein (COMP) in Tendon and Its Variation With Tendon Site, Age and Load," Matrix Biol., **16**(5), pp. 255–271.
[117] Thorpe, C. T., Birch, H. L., Clegg, P. D., and Screen, H. R. C., 2013, "The Role of the Non-Collagenous Matrix in Tendon Function," Int. J. Exp. Pathol., **94**(4), pp. 248–259.
[118] Zitnay, J. L., and Weiss, J. A., 2018, "Load Transfer, Damage, and Failure in Ligaments and Tendons," J. Orthop. Res., **36**(12), pp. 3093–3104.
[119] Taye, N., Karoulias, S. Z., and Hubmacher, D., 2020, "The 'Other' 15–40%: The Role of Non-Collagenous Extracellular Matrix Proteins and Minor Collagens in Tendon," J. Orthop. Res., **38**(1), pp. 23–35.

[120] Woo, S. L.-Y., Hollis, J. M., Adams, D. J., Lyon, R. M., and Takai, S., 1991, "Tensile Properties of the Human Femur-Anterior Cruciate Ligament-Tibia Complex: The Effects of Specimen Age and Orientation," Am. J. Sports Med., **19**(3), pp. 217–225.
[121] Pardes, A. M., Beach, Z. M., Raja, H., Rodriguez, A. B., Freedman, B. R., and Soslowsky, L. J., 2017, "Aging Leads to Inferior Achilles Tendon Mechanics and Altered Ankle Function in Rodents," J. Biomech., **60**, pp. 30–38.
[122] Muench, J. R., Thelen, D. G., and Henak, C. R., 2020, "Interfibrillar Shear Behavior Is Altered in Aging Tendon Fascicles," Biomech. Model. Mechanobiol., **19**(3), pp. 841–849.
[123] Svensson, R. B., Heinemeier, K. M., Couppé, C., Kjaer, M., and Magnusson, S. P., 2016, "Effect of Aging and Exercise on the Tendon," J. Appl. Physiol., **121**(6), pp. 1353–1362.
[124] Fung, D. T., Wang, V. M., Laudier, D. M., Shine, J. H., Basta-Pljakic, J., Jepsen, K. J., Schaffler, M. B., and Flatow, E. L., 2009, "Subrupture Tendon Fatigue Damage," J. Orthop. Res., **27**(2), pp. 264–273.
[125] Arya, S., and Kulig, K., 2010, "Tendinopathy Alters Mechanical and Material Properties of the Achilles Tendon," J. Appl. Physiol., **108**(3), pp. 670–675.
[126] Thorpe, C. T., Udeze, C. P., Birch, H. L., Clegg, P. D., and Screen, H. R. C., 2012, "Capacity for Sliding Between Tendon Fascicles Decreases With Ageing in Injury Prone Equine Tendons: A Possible Mechanism for Age-Related Tendinopathy?," Eur. Cells Mater., **25**, pp. 48–60.
[127] Tom, S., Parkinson, J., Ilic, M. Z., Cook, J., Feller, J. A., and Handley, C. J., 2009, "Changes in the Composition of the Extracellular Matrix in Patellar Tendinopathy," Matrix Biol., **28**(4), pp. 230–236.
[128] Plaas, A., Sandy, J. D., Liu, H., Diaz, M. A., Schenkman, D., Magnus, R. P., Bolam-Bretl, C., Kopesky, P. W., Wang, V. M., and Galante, J. O., 2011, "Biochemical Identification and Immunolocalizaton of Aggrecan, ADAMTS5 and Inter-Alpha-Trypsin-Inhibitor in Equine Degenerative Suspensory Ligament Desmitis," J. Orthop. Res., **29**(6), pp. 900–906.
[129] Thorpe, C. T., Peffers, M. J., Simpson, D., Halliwell, E., Screen, H. R. C., and Clegg, P. D., 2016, "Anatomical Heterogeneity of Tendon: Fascicular and Interfascicular Tendon Compartments Have Distinct Proteomic Composition," Sci. Rep., **6**(1), p. 20455.
[130] Andarawis-Puri, N., Flatow, E. L., and Soslowsky, L. J., 2015, "Tendon Basic Science: Development, Repair, Regeneration, and Healing," J. Orthop. Res., **33**(6), pp. 780–784.
[131] Caves, J. M., Kumar, V. A., Xu, W., Naik, N., Allen, M. G., and Chaikof, E. L., 2010, "Microcrimped Collagen Fiber-Elastin Composites," Adv. Mater., **22**(18), pp. 2041–2044.
[132] Caliari, S. R., Ramirez, M. A., and Harley, B. A. C., 2011, "The Development of Collagen-GAG Scaffold-Membrane Composites for Tendon Tissue Engineering," Biomaterials, **32**(34), pp. 8990–8998.
[133] Kishore, V., Paderi, J. E., Akkus, A., Smith, K. M., Balachandran, D., Beaudoin, S., Panitch, A., and Akkus, O., 2011, "Incorporation of a Decorin Biomimetic Enhances the Mechanical Properties of Electrochemically Aligned Collagen Threads," Acta Biomater., **7**(6), pp. 2428–2436.
[134] Jaiswal, D., Yousman, L., Neary, M., Fernschild, E., Zolnoski, B., Katebifar, S., Rudraiah, S., Mazzocca, A., and Kumbar, S., 2020, "Tendon Tissue Engineering: Biomechanical Considerations," Biomed. Mater., **15**(5), p. 052001.
[135] Cheng, C. W., Solorio, L. D., and Alsberg, E., 2014, "Decellularized Tissue and Cell-Derived Extracellular Matrices as Scaffolds for Orthopaedic Tissue Engineering," Biotechnol. Adv., **32**(2), pp. 462–484.
[136] Thorpe, C. T., Godinho, M. S. C., Riley, G. P., Birch, H. L., Clegg, P. D., and Screen, H. R. C., 2015, "The Interfascicular Matrix Enables Fascicle Sliding and Recovery in Tendon, and Behaves More Elastically in Energy Storing Tendons," J. Mech. Behav. Biomed. Mater., **52**, pp. 85–94.
[137] Zamboulis, D. E., Thorpe, C. T., Kharaz, Y. A., Birch, H. L., Screen, H. R. C., and Clegg, P. D., 2020, "Postnatal Mechanical Loading Drives Adaptation of Tissues Primarily Through Modulation of the Non-Collagenous Matrix," eLife, **9**, pp. 1–23.
[138] Thorpe, C. T., Udeze, C. P., Birch, H. L., Clegg, P. D., and Screen, H. R. C., 2012, "Specialization of Tendon Mechanical Properties Results From Interfascicular Differences," J. R. Soc. Interface, **9**(76), pp. 3108–3117.
[139] Thorpe, C. T., Karunaseelan, K. J., Ng Chieng Hin, J., Riley, G. P., Birch, H. L., Clegg, P. D., and Screen, H. R. C., 2016, "Distribution of Proteins Within Different Compartments of Tendon Varies According to Tendon Type," J. Anat., **229**(3), pp. 450–458.
[140] Thorpe, C. T., Riley, G. P., Birch, H. L., Clegg, P. D., and Screen, H. R. C., 2016, "Fascicles and the Interfascicular Matrix Show Adaptation for Fatigue Resistance in Energy Storing Tendons," Acta Biomater., **42**, pp. 308–315.
[141] Fung, D. T., Wang, V. M., Andarawis-Puri, N., Basta-Pljakic, J., Li, Y., Laudier, D. M., Sun, H. B., Jepsen, K. J., Schaffler, M. B., and Flatow, E. L., 2010, "Early Response to Tendon Fatigue Damage Accumulation in a Novel In Vivo Model," J. Biomech., **43**(2), pp. 274–279.
[142] Herod, T. W., and Veres, S. P., 2017, "Development of Overuse Tendinopathy: A New Descriptive Model for the Initiation of Tendon Damage During Cyclic Loading," J. Orthop. Res., **36**(1), pp. 467–476.
[143] Zitnay, J. L., Seob Jung, G., Lin, A. H., Qin, Z., Li, Y., Michael Yu, S., Buehler, M. J., and Weiss, J. A., 2020, "Accumulation of Collagen Molecular Unfolding Is the Mechanism of Cyclic Fatigue Damage and Failure in Collagenous Tissues," Sci. Adv., **6**(35), pp. 1–10.
[144] Shepherd, J. H., Riley, G. P., and Screen, H. R. C., 2014, "Early Stage Fatigue Damage Occurs in Bovine Tendon Fascicles in the Absence of Changes in Mechanics at Either the Gross or Micro-Structural Level," J. Mech. Behav. Biomed. Mater., **38**, pp. 163–172.
[145] Starborg, T., Lu, Y., Huffman, A., Holmes, D. F., and Kadler, K. E., 2009, "Electron Microscope 3D Reconstruction of Branched Collagen Fibrils In Vivo," Scand. J. Med. Sci. Sports, **19**(4), pp. 547–552.
[146] Svensson, R. B., Herchenhan, A., Starborg, T., Larsen, M., Kadler, K. E., Qvortrup, K., and Magnusson, S. P., 2017, "Evidence of Structurally Continuous Collagen Fibrils in Tendons," Acta Biomater., **50**, pp. 293–301.
[147] Sartori, J., and Stark, H., 2021, "Tracking Tendon Fibers to Their Insertion—A 3D Analysis of the Achilles Tendon Enthesis in Mice," Acta Biomater., **120**, pp. 146–155.
[148] Provenzano, P. P., and Vanderby, R., 2006, "Collagen Fibril Morphology and Organization: Implications for Force Transmission in Ligament and Tendon," Matrix Biol., **25**(2), pp. 71–84.
[149] Safa, B. N., Peloquin, J. M., Natriello, J. R., Caplan, J. L., and Elliott, D. M., 2019, "Helical Fibrillar Microstructure of Tendon Using Serial Block-Face Scanning Electron Microscopy and a Mechanical Model for Interfibrillar Load Transfer," J. R. Soc. Interface, **16**(160), p. 20190547.
[150] Wunderli, S. L., Blache, U., and Snedeker, J. G., 2020, "Tendon Explant Models for Physiologically Relevant In Vitro Study of Tissue Biology—A Perspective," Connect. Tissue Res., **61**(3–4), pp. 262–277.
[151] Kelwick, R., Desanlis, I., Wheeler, G. N., and Edwards, D. R., 2015, "The ADAMTS (a Disintegrin and Metalloproteinase With Thrombospondin Motifs) Family," Genome Biol., **16**(1), p. 113.