Structure-Function Relationships of Postnatal Tendon Development: A Parallel to Healing.

ARTICLE *in* MATRIX BIOLOGY: JOURNAL OF THE INTERNATIONAL SOCIETY FOR MATRIX BIOLOGY · JANUARY 2013

Impact Factor: 5.07 · DOI: 10.1016/j.matbio.2013.01.007 · Source: PubMed

3 AUTHORS, INCLUDING:

Brianne Connizzo
University of Pennsylvania
22 PUBLICATIONS **162** CITATIONS

SEE PROFILE

Matrix Biology 32 (2013) 106–116

Contents lists available at SciVerse ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

Mini review

Structure–function relationships of postnatal tendon development: A parallel to healing

Brianne K. Connizzo, Sarah M. Yannascoli, Louis J. Soslowsky *

McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA, USA

ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form 2 January 2013
Accepted 2 January 2013

Keywords:
Tendon
Development
Healing
Structure
Extracellular matrix
Tissue engineering

ABSTRACT

This review highlights recent research on structure–function relationships in tendon and comments on the parallels between development and healing. The processes of tendon development and collagen fibrillogenesis are reviewed, but due to the abundance of information in this field, this work focuses primarily on characterizing the mechanical behavior of mature and developing tendon, and how the latter parallels healing tendon. The role that extracellular matrix components, mainly collagen, proteoglycans, and collagen cross-links, play in determining the mechanical behavior of tendon will be examined in this review. Specifically, collagen fiber re-alignment and collagen fibril uncrimping relate mechanical behavior to structural alterations during development and during healing. Finally, attention is paid to a number of recent efforts to augment injured tendon and how future efforts could focus on recreating the important structure–function relationships reviewed here.

Contents

* Corresponding author.
E-mail address: soslowsk@mail.med.upenn.edu (L.J. Soslowsky).

0945-053X/$ – see front matter
http://dx.doi.org/10.1016/j.matbio.2013.01.007

1. Introduction

The ability of tendon to provide both rigidity and flexibility is based on its nonlinear, viscoelastic, anisotropic, and heterogeneous mechanical properties. These functional properties are derived from the complex structure of the extracellular matrix, which is comprised mostly of collagen bundled into progressively larger subunits. Here we review current literature on structure–function relationships of tendon in adulthood and throughout postnatal development, using the latter to draw connections to the healing tendon to envision new strategies for tendon repair augmentation. We begin by reviewing the structure, composition, and function of mature tendon, followed by the development and assembly of tendon in postnatal animal models. We then discuss the structural response to load in adult tendon and how that response is altered in postnatal development. Finally, we examine structural alterations in healing tendon and the connection between healing and development. We conclude with a commentary on current augmentation strategies and provide insight into possible future directions.

2. Mature tendon structure and function

Tendons are fibrous, dense, soft tissue structures that serve as a conduit for the efficient transfer of energy through muscle contraction, acting to both guide movement and stabilize the skeleton. Damage to these structures affects the balance between stability and mobility, thus altering joint kinematics and ultimately leading to destruction of the joint (Woo et al., 2005). Understanding the basics of mature tendon microstructure and mechanical function is essential to re-establishing these attributes in the injured tendon.

2.1. Structure and composition

Tendons contain a range of morphologically diverse fibrous soft tissue structures that connect muscle to bone. Their shape ranges from long and round to short and flat, allowing them to not only transmit muscle-generated force to bone, but also to store elastic energy, to serve as an articulating surface, or to constitute a pulley mechanism. The morphology of each tendon is derived by its unique mechanical environment; tendons with a more rounded morphology undergo uniaxial tensile loads and exhibit more closely aligned, parallel bundles of collagen fibers (e.g., flexor tendons of the hand or foot), while tendons with a more flattened shape endure more complex loading and exhibit more diversely oriented collagen fibers (e.g. rotator cuff tendons) (Einhorn et al., 2007). This collagen orientation promotes very high strength in the direction of fiber alignment, which is dependent on the underlying organizational structure of collagen within the extracellular matrix (Killian et al., 2012).

Tendon is organized in a hierarchical manner, with type I collagen oriented parallel to the mechanical axis. The assembly begins with three intracellular peptide alpha chains (two alpha1 chains, and one alpha2 chain) that organize into a procollagen triple helix structure. This structure is then transported outside the cell where it is cleaved into tropocollagen, which covalently bonds with the surrounding matrix. This chemical alteration yields insoluble collagen molecules which then aggregate neatly to form dense microfibrils. Microfibrils further organize by lateral and longitudinal stacking, forming a moderately twisted, lattice-type configuration, called a fibril (Fig. 1). Fibrils continue to pack together to form larger fibers, which then further combine to form fascicles. The bundling of fascicles by connective tissue sheaths known as endotenon (intrasubstance), and epitenon (circumferential to the tendon fascicles), is what forms a full tendon (Killian et al., 2012).

The underlying microstructure of tendon is characterized by a few cells located within this highly organized extracellular matrix. The majority of the matrix is comprised of type I collagen (95%), however, 5% of mature adult tendon is comprised of minor amounts of additional collagens, namely types III and V (Birk and Mayne, 1997). Overall, dehydrated tendon is approximately 70–80% type I collagen, with the remaining 20–30% of tendon weight consisting of minor collagens, elastin, proteoglycans, glycolipids, and cellular material (Woo et al., 2005). Proteoglycans represent a diverse group of macromolecules which regularly attach to collagen fibrils in an orthogonal manner (Fig. 1) (Scott et al., 1981; Cribb and Scott, 1995). The most predominant proteoglycan associated with tendon is decorin, however, hyaluronan, biglycan, fibromodulin, and lumican also are present in small amounts (Iozzo and Murdoch, 1996; Derwin et al., 2001). These proteoglycans have specialized carbohydrate chains known as glycosaminoglycans which carry a highly negative charge that attracts and binds water molecules. In its natural state, 65–70% of a tendon's total weight is water (Woo et al., 2005). The synthesis of all these matrix components is performed by the tendon fibroblast, which accounts for 90–95% of cells within a tendon (Franchi et al., 2007b). This orchestration of tendon assembly is coordinated by elaborate networks of fibroblast cytoplasmic processes which extend within the matrix to connect with nearby cells via gap junctions (McNeilly et al., 1996; Benjamin and Ralphs, 2000).

2.2. Tendon to bone insertion

Although tendon appears homogeneous on gross macroscopic inspection, there is great mechanical, histological, and compositional diversity. The properties we have outlined in this review thus far concern the midsubstance of the tendon. However, the junction where tendon meets bone, known as the enthesis or insertion site, is a highly specialized tissue interface of great clinical importance, thus necessitating special attention. The enthesis is a particularly complex attachment where a flexible and rope-like tendon (200 MPa tensile modulus) joins rigid and stiff bone (20 GPa tensile modulus) (Orthopaedic Basic Science). To accommodate the stress and strain concentrations which arise at the junction of two different materials, the enthesis is composed of a transitional tissue with graded morphology and mechanobiology.

The enthesis may take two forms, fibrous or fibrocartilaginous. Fibrous entheses, also known as indirect insertions, are characterized by the incorporation of tendon to the periosteum during development with eventual direct insertion to the metaphysis or diaphysis of long bones by maturation. Fibrocartilaginous, or direct insertions, are characterized by the direct incorporation of the tendon to the epiphysis or apophysis of bone via a complex transitional tissue (Fig. 1). Under optical microscopy, the four transitional zones can be identified as: (1) tendon proper, (2) fibrocartilage, (3) mineralized fibrocartilage, and (4) bone (Benjamin et al., 2002). Zone 1 represents tendon proper with predominant hierarchical collagen type I organization, analogous to the tendon midsubstance composition. Zone 2 represents the unmineralized fibrocartilaginous tissue where the predominant collagen becomes types II and III and the proteoglycan decorin is now accompanied by aggrecan. Zone 3 marks the mineralized fibrocartilaginous zone with the predominance of collagens II and X, accompanied by aggrecan and the gradual accumulation of minerals. By zone 4, the tissue is consistent with bone where collagen I again predominates along with a very dense mineral content (Thomopoulos et al., 2010). These zones thus represent a gradual transition of compositional, biochemical, and structural elements, rather than distinct zones with sharp boundaries (Wopenka et al., 2008). Staining of the insertion site tissue highlights a dense basophilic border known as the “tidemark,” or boundary between unmineralized and mineralized tissue (Benjamin et al., 1986). Historically many understood the tidemark to simply represent the boundary between hard and soft tissue. Further research has now demonstrated a complex transition in matrix gene expression (from tendinous to cartilaginous), angular deviation between fibers (decreased fiber alignment and organization from tendon to bone), and mineral concentration (linearly

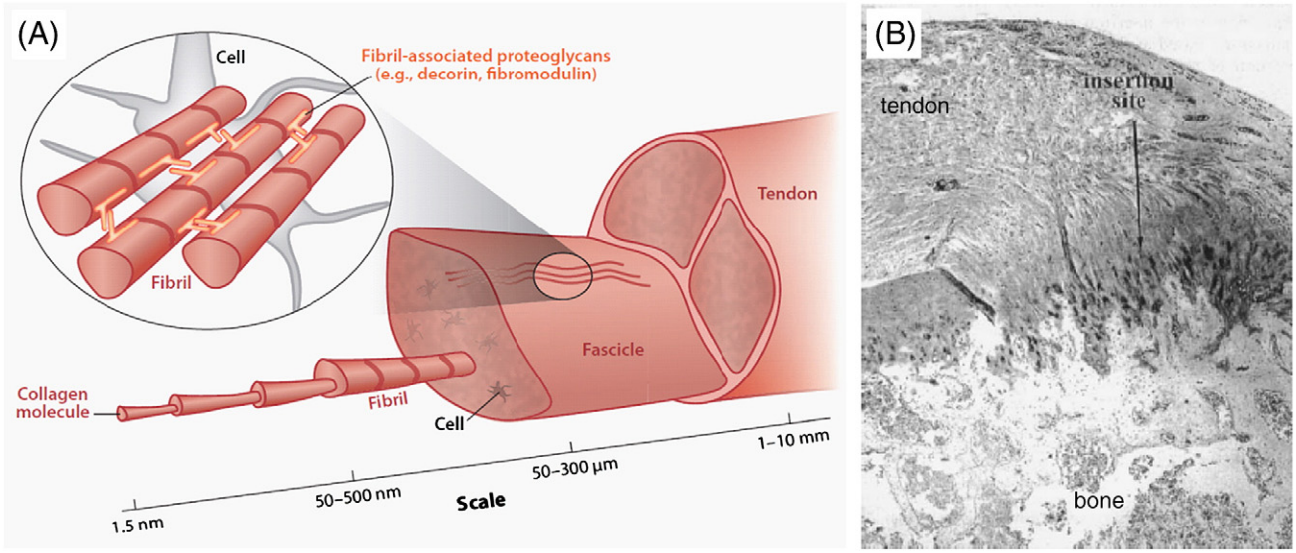

Fig. 1. (A) Hierarchical structure of tendon spanning from the single collagen molecule up to fibrils, fascicles, and whole tendon. Inset image describes the structure of fibril-associated proteoglycans, the most abundant of which is decorin in tendon. (B) Histological section (4×) of the rat supraspinatus tendon-to-bone insertion site, highlighting the transition zone.
Panel A: Image reproduced from Voleti et al. (2012). Panel B: Image reproduced from Thomopoulos et al. (2002).

increasing from tendon to bone) (Thomopoulos et al., 2003; Wopenka et al., 2008; Genin et al., 2009). Clearly, this transition zone undergoes dramatic alterations along its length, which likely account for the efficient transfer of forces across tissues of different material properties. Despite the insertion site's unique ability to reduce stress concentrations and outward splay of the tissue under particularly high stress (Thomopoulos et al., 2006), injury at these sites is frequent. Clinically, the reattachment of these dissimilar tissues has proved that simple re-approximation does not recreate this complex transitional tissue.

2.3. Function

Tendon is a compliant, anisotropic material which has a high modulus (slope of the linear region of the stress–strain curve) under tension, but collapses under compression. It exhibits nonlinear biomechanical behavior as exhibited by a typical stress–strain curve with an initial, non-linear "toe-region" with low stiffness, followed by the "linear-region" with increased stiffness (Fig. 2). Clinically, these properties allow for tendon to both guide movement (low stiffness) and provide stability (high stiffness). When viewed under light microscopy tendon exhibits a periodic waveform configuration, known as "crimp." Many have implicated the straightening of tendon crimp as the primary etiology of the non-linear behavior demonstrated under low tensile loads (Diamant et al., 1972; Atkinson et al., 1999).

In addition to their anisotropic, non-linear behavior, tendons exhibit viscoelastic properties identified as stress relaxation, hysteresis, and creep. Stress relaxation refers to a non-linear decrease in stress over a period of time when a tendon is held under constant tension. This process is both static and dynamic, as demonstrated by a similar decrease in peak stress over time with repetitive, cyclic tensile loading. Hysteresis represents the energy loss within the tendon with dynamic testing, accounting for a gradual change in load-elongation curves with tendon loading and unloading. Creep occurs when a tendon is held under constant tension, and a measurable increase in tendon length over time is observed. It is thought that creep represents the progressive fiber recruitment by tendon. These viscoelastic properties emphasize the ability of tendon to structurally adapt to constant or cyclical loads in order to reach biomechanical equilibrium (Einhorn et al., 2007). In addition to viscoelasticity, tendon also exhibits strain rate sensitivity, meaning that the mechanical behavior of the tissue is dependent on the speed at which the tendon is strained.

3. Development of the extracellular matrix

Tendon is a complex tissue exhibiting nonlinear mechanical behavior and its function is highly dependent on the structure and composition of the tissue. The extracellular matrix is an integral component of tendon's ability to withstand mechanical loads, including at the insertion site where a highly compliant tissue merges with a very stiff tissue (bone). In order to understand how this tissue works, we must first understand how it is assembled and how it is connected.

3.1. Embryogenesis

Although the process of tendon's embryonic structural development varies by location and type of tendon, it generally takes place over the course of three main stages: induction, organization, and aggregation or differentiation (Deries et al., 2010). In the first stage, tendon progenitor cells originate from the primary germ layer compartments along with progenitors for cartilage and muscle (Maeda et al., 2011). This process is different for longer limb tendons as opposed to short trunk tendons. Trunk tendon progenitor cells arise from the sclerotome in a domain that is distinct from that of cartilage cells, called the syndetome (Tozer and Duprez, 2005). Recent data from both chick and mouse show that the myotome (where myogenic cells are) is required for the formation of tendon progenitor cells. Removal of the myotome leads to the loss of the tendon-specific marker scleraxis (*Scx*) (Brent et al., 2005). In contrast to the trunk tendons, the limb tendon progenitor cells are mixed in with cartilage cells in the limb bud and development of these cells in the limb is muscle-independent. The development of limb tendon and muscle is also much more spread out in time as the limb bud is growing and developing simultaneously (Tozer and Duprez, 2005). Following the induction of tendon progenitors, cells undergo organization by aligning in parallel bundles along the long axis of where the tendon will form. Finally, in the third stage, these cells differentiate into proper tendon cells, or tenocytes.

There have been many studies investigating the roles of various signaling cascades in tendon embryogenesis. Induction of the tendon progenitor cells from the myotome has been associated with fibroblast growth factor (FGF) signaling in both limb and trunk tendons. Recent studies have showed that FGF signaling is both necessary and sufficient for induction of the syndetome, and that FGF induces signaling of *Scx* as well as other tendon markers (Schweitzer et al., 2010). However,

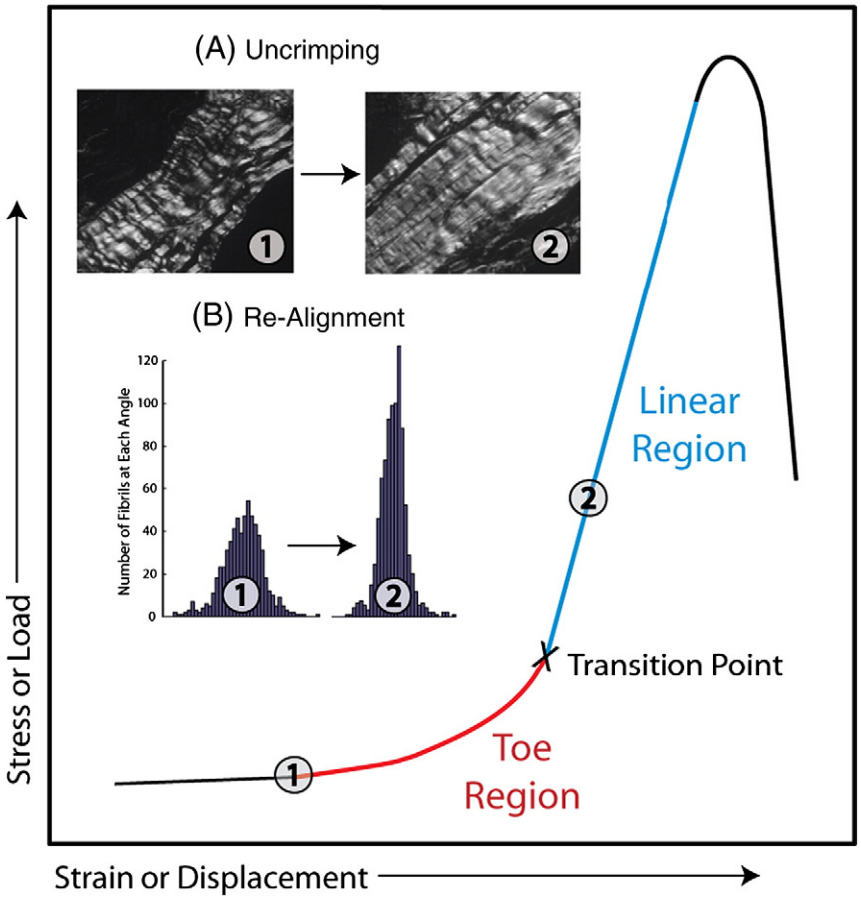

Fig. 2. Typical stress–strain curve of tendon shows an elongated toe region with a low stiffness transitioning to a high stiffness linear region. Insets are two mechanisms of tendon's response to load within the toe region, notably (A) the uncrimping of collagen fibrils, and (B) the re-alignment of collagen fibrils, represented by a decrease in the variance of the distribution of fiber angles from the toe to the linear region.

inhibition of FGF signaling in mouse embryos resulted in a loss of *Scx* expression but no specific tendon disruption, suggesting other molecules contribute to the induction of tendon progenitor cells. In addition, transforming growth factor-beta (TGF-β) signaling has been associated with the organization phase of embryogenesis (Schweitzer et al., 2010). Disruption of TGF-β signaling results in the loss of all tendon tissue and studies show that TGF-β plays a role in regulating *Scx* expression and in determining cell fate during organization (Pryce et al., 2009). Finally, there has been recent evidence that the role of *Scx* during embryonic development is not fully elucidated (Deries et al., 2010). *Scx*-deficient mice exhibit normal initial induction and alignment of tendon progenitors but a major loss of force-transmitting tendons later, suggesting that more cues are necessary to direct appropriate tissue deposition (Schweitzer et al., 2010). *Scx* may play a bigger role during aggregation and differentiation than during induction, especially for limb tendons (Murchison et al., 2007).

In addition to molecular signaling, mechanical loading also contributes to the appropriate formation of tendon during embryogenesis, but only at later stages. Tendon progenitor cells undergo major dynamic reorganization and align between muscles and cartilage during the second and third stages (Schweitzer et al., 2010). This organization has been mimicked by slowly stretching tissue in vitro, suggesting that muscle-induced loading could play a key role in this developmental process (Kalson et al., 2011). Removal of mechanical loading results in a reduction of *Scx* expression, indicating a relationship between mechanical loading and signaling during embryonic development (Schweitzer et al., 2010; Maeda et al., 2011). The relationship between load and structural change can be seen during development, both embryonic and post-natal, as well as during healing.

3.2. Fibrillogenesis

Once tendon progenitors undergo aggregation and become distinct tendon units, they begin to lay down collagenous matrix in the form of small diameter fibrils (Liu et al., 2010). This is the beginning of the hierarchical assembly of collagen in tendon, known as fibrillogenesis. Fibrillogenesis continues after birth with the assembly of collagen I molecules, followed by linear and lateral growth (Birk et al., 1995, 1997; Zhang et al., 2005). This process allows collagen molecules to assemble into its ultimate hierarchical fashion (Fig. 1). First, collagen molecules assemble to form immature fibril intermediates (discussed in Section 2.1). The formation of immature fibril intermediates can be influenced at a number of points, including packaging for secretion, procollagen processing, and collagen interactions with proteins such as other collagens and proteoglycans. Following this molecular assembly, collagen fibril intermediates assemble end-to-end to form longer fibrils consistent with mature, mechanically functional fibrils. They then associate laterally to generate larger fibril diameters (Zhang et al., 2005). These two processes result in a large distribution of fibril diameters in adult tendon. The process of linear and lateral growth is regulated by a number of molecules, including minor collagens such as collagens III, V, XI, XII, and XIV, and proteoglycans.

3.3. Minor collagens

There are several secondary collagens present within tendon which act to regulate fibrillogenesis and thus contribute to the structural characteristics of the matrix. Collagen III has also been implicated during development as it has been associated with changes in fibril diameter,

specifically with small diameter, immature fibrils (Tozer and Duprez, 2005; Zhang et al., 2005). Collagen III expression decreases gradually during development so its high expression early on suggests a role in initial fibril assembly (Tozer and Duprez, 2005). While collagen III has not been studied extensively in tendon development, the expression of collagen III is elevated after injury and this collagen could play a role in the healing process, perhaps with post-injury fibrillogenesis (Shirachi et al., 2011).

Both collagens V and XI form a heterotypic collagen fibril that works in conjunction with collagens I and II to regulate fibril assembly (Hansen et al., 2002). In vitro studies have demonstrated that collagens I and II that are associated with collagens V and XI have smaller diameter fibrils, suggesting that these collagens regulate fibril diameter during growth and development (Birk, 2001; Segev et al., 2006). *Col5a1* deletion is a lethal phenotype by embryonic day 10 (Wenstrup et al., 2004). However, *Col5a1* heterozygous mice are used to demonstrate the Ehlers–Danlos syndrome phenotype, thus demonstrating intact fibril structure with decreased tendon diameter and increased tissue elasticity (Wenstrup et al., 2011). By combining *Col5a1* and *Col11a1* mutations, experiments implicate the role of collagen V in nucleation of fibrils in synergy with fibril assembly properties of collagen XI. These genetically altered mice express severe fibril structure deformations suggesting the interaction of these two minor collagens (Wenstrup et al., 2011).

Collagens XII and XIV have been similarly implicated to possess a synergistic regulatory role during collagen fibrillogenesis. These collagens have been labeled as fibril-associated collagens with interrupted triple helices (FACIT) collagens due to their interaction with collagen I. Collagen XII has been postulated to function by integrating adjacent matrix components through its ability to bind the proteoglycans decorin and fibromodulin as well as interact with collagen I fibrils (Zhang et al., 2005). While collagen XIV has been shown to integrate fibrils into fibers during development, collagen XII performs similar actions, but has increased prevalence in mature tendon. This has led some to hypothesize that collagen XII replaces collagen XIV both functionally and structurally in later development (Ansorge et al., 2009).

3.4. Proteoglycans during development

Proteoglycans also play a key role during assembly of collagen and development of the extracellular matrix. Small leucine-rich proteoglycans (SLRPs), specifically class I (biglycan and decorin) and class II (fibromodulin and lumican), are regulators of fibril assembly as they can delay fibril formation (Svensson et al., 1999; Zhang et al., 2006; Kalamajski and Oldberg, 2010). Decorin is the most prevalent proteoglycan in tendon and can influence both fibril diameter and shape during assembly by restricting uncontrolled lateral growth (constraining fibrils to a uniform, thin diameter profile) (Kalamajski and Oldberg, 2010). Mouse skin and tail tendon lacking decorin have irregular fibril profiles, with larger diameter fibrils, abnormal lateral fusion and less regulated assembly. Decorin expression is high throughout development but peaks at 4 and 10 days postnatal in mice, suggesting that it plays a role in lateral fibril growth (Zhang et al., 2006). Biglycan also inhibits fibrillogenesis of collagen in vitro however its relationship with collagen is not well characterized. Altered collagen fibril formation and tissue organization is noted in fibromodulin-null mice. The Achilles tendons of these mice have irregular cross-sections of fibrils and thinner fibrils on average, suggesting fibromodulin's role of controlling fibril thickness (Svensson et al., 1999). Fibromodulin may also play a role in connecting fibrils into fibers. While lumican is believed to modulate fiber thickening early in development, fibromodulin modulates thick fiber formation during the later stages of growth (Kalamajski and Oldberg, 2010).

SLRPs may also play a role in regulating the intermolecular cross-linking of collagen during development. While no key mechanism for regulation of cross-linking has been found yet, SLRPs can either act as a co-receptor for lysyl oxidase or sterically hinder the binding of lysyl oxidase to improper lysines. Since SLRPs regulate fibril formation, they could also align collagens in a way to allow oxidized lysines to react with lysines on adjacent fibrils, thus creating interfibrillar links (Kalamajski and Oldberg, 2010). In vitro, the addition of decorin to decorin-deficient fibroblasts increased collagen cross-link formation (Seidler et al., 2005). However, this relationship has not been studied extensively and more investigation into possible connections between SLRPs and intermolecular cross-linking is warranted.

4. Adult response to load

The matrix synthesized by tendon fibroblasts is the principal component of tendon, thus dominating the tissue's properties with respect to biomechanical behavior. When loaded under uniaxial tension in the direction of predominant collagen alignment, the matrix components structurally contribute to the characteristic non-linear, anisotropic, and viscoelastic behavior as described previously. This response of tendon to the application of load is defined by its microstructural behavior. Not only does this rely on the mechanical strength of collagen fibrils, fibers and fascicles, but also on fibril–fibril interactions (collagen re-alignment, uncrimping), fibril–proteoglycan interactions, and molecular interactions (collagen cross-linking).

4.1. Collagen structural mechanics

A number of researchers have studied the mechanical properties and behavior of fibrils themselves (van der Rijt et al., 2006; Heim et al., 2007; Svensson et al., 2010; Gautieri et al., 2011, 2012; Veres and Lee, 2012). Collagen fibrils have been likened to nanoscale biological cables, and with techniques such as atomic force microscopy, the isolated study of their mechanical properties is possible. The study of microfibril behavior demonstrated that key deformations during mechanical loading occurred via the straightening of collagen's triple helix structure, followed by axial stretching, and eventual molecular uncoiling (Gautieri et al., 2011). Furthermore, the mechanical overload of bovine tail tendons found that triple helix uncoiling occurred at discrete, repeated locations along the length of the fibril, creating kinked regions in previously straight fibrils, thus suggesting that tissue failure is distributed longitudinally along the entire length of a fibril (Veres and Lee, 2012). Even further analysis of collagen microstructure with the atomistic modeling of a single collagen-like peptide molecule found that the collagen molecule exhibited dramatically reduced viscosity compared to fibrils and fiber bundles (Gautieri et al., 2012). Thus, the mechanical behavior of the collagen fibrils themselves has not been able to account for full tendon mechanics. A previous study of fibril mechanics has revealed that the characteristic non-linear behavior of these structures was dependent on immersion of the fibrils in an aqueous solution, while linear behavior was exhibited with fibril testing at ambient temperatures. This suggested that the viscoelastic behavior could be exhibited at the individual fibril level, but that water was a crucial component (van der Rijt et al., 2006). The viscous behavior of collagen has been attributed to hydroplaning of interstitial water within subfibrillar elements, causing decreased friction and viscous behavior (Silver et al., 2002), thus consistent with these results. This further supports the theory that elasticity is the result of collagen triple helix stretching, while viscosity is the result of a hydrated matrix and other fibril–molecular and intermolecular interactions (van der Rijt et al., 2006; Gautieri et al., 2012).

Furthermore, recent studies have addressed the quasi-static mechanical behavior of tendon. One study measured the mechanical properties of rat tail tendon fascicles and seven structural and compositional variables, attempting to find evidence of direct structure–function relationships using a multiple regression model (Robinson et al., 2004a). The most prevalent predictors of mechanical behavior in this study were GAG content and collagen fibril area fraction.

Interestingly, mean collagen fibril diameter was not a significant predictor. This contrasts several studies where fibril diameter was correlated with mechanical properties, (Parry, 1988; Derwin and Soslowsky, 1999; Hansen et al., 2010) but agrees with other studies observing the lack of correlation in developing tendon or self-assembled collagen fibers (Birk et al., 1991; Christiansen et al., 2000; Hansen et al., 2010). A study in human patellar tendon found differences in collagen fibril distribution between the posterior and anterior aspect of the tendon, but no correlation with fascicle mechanical properties (Fig. 3) (Hansen et al., 2010). Another study investigated fibril morphology and mechanical properties in the Achilles tendon, noting that larger fibrils may be associated with a stiffer tendon but that this could be due to fibril–fibril interactions or fibril–non-fibrillar matrix interactions (Rigozzi et al., 2010). Similarly, a few groups have sought to determine whether fibrils are continuous or discontinuous along the length of the tendon, and subsequently to elucidate the mechanism of load transfer within these tendons. One study used electron microscopy to visualize collagen fibrils and speculate on this continuity, arguing that fibrils must be continuous along the length (as opposed to collagen segments linked by interfibrillar bridges such as proteoglycans) because very few fibril ends are visualized (Provenzano and Vanderby, 2006). However, since the mechanical behavior of the tendon cannot be explained by collagen fibrils alone as stated above, this suggests that there are alternative mechanisms of load transfer such as fibril re-alignment, uncrimping, sliding, stress transfer through the non-fibrillar matrix, etc.

4.2. Re-alignment of collagen fibers

When tendon is being loaded, collagen fibers shift their orientation towards the axis of loading, decreasing the distribution of fiber angles (Fig. 2). This mechanism is called collagen fiber re-alignment. The native collagen alignment of the tissue varies along the length of a tendon, with a more disorganized fiber matrix located at the tendon-to-bone insertion (Lake et al., 2009, 2010). The location-dependent alignment plays a role in both the mechanical properties as well as the re-alignment that occurs with loading. Recent efforts in our lab focused on the ability to quantify collagen fiber re-alignment using a novel polarized light setup that allows for simultaneous measurement and mechanical testing (Lake et al., 2009, 2010; Miller et al., 2012a,b, 2012d). This approach has determined that re-alignment occurs differently when measured at various points throughout the mechanical test. In addition, measurement of local re-alignment has determined that changes in collagen fiber alignment throughout loading is location-dependent (Miller et al., 2012a,b, 2012d). This has also allowed us to determine effects of preconditioning, which is commonly used to provide a consistent "mechanical history" across all testing samples and thus reduce variability in the data. Fiber re-alignment in the midsubstance of tendon has been speculated as one explanation implicated in the biomechanical changes seen from preconditioning (Sverdlik and Lanir, 2002; Quinn and Winkelstein, 2011; Miller et al., 2012b, 2012d). Using rat supraspinatus, preconditioning was found to cause the largest amount of collagen re-alignment, while smaller, yet significant re-alignment was demonstrated within both the toe and linear

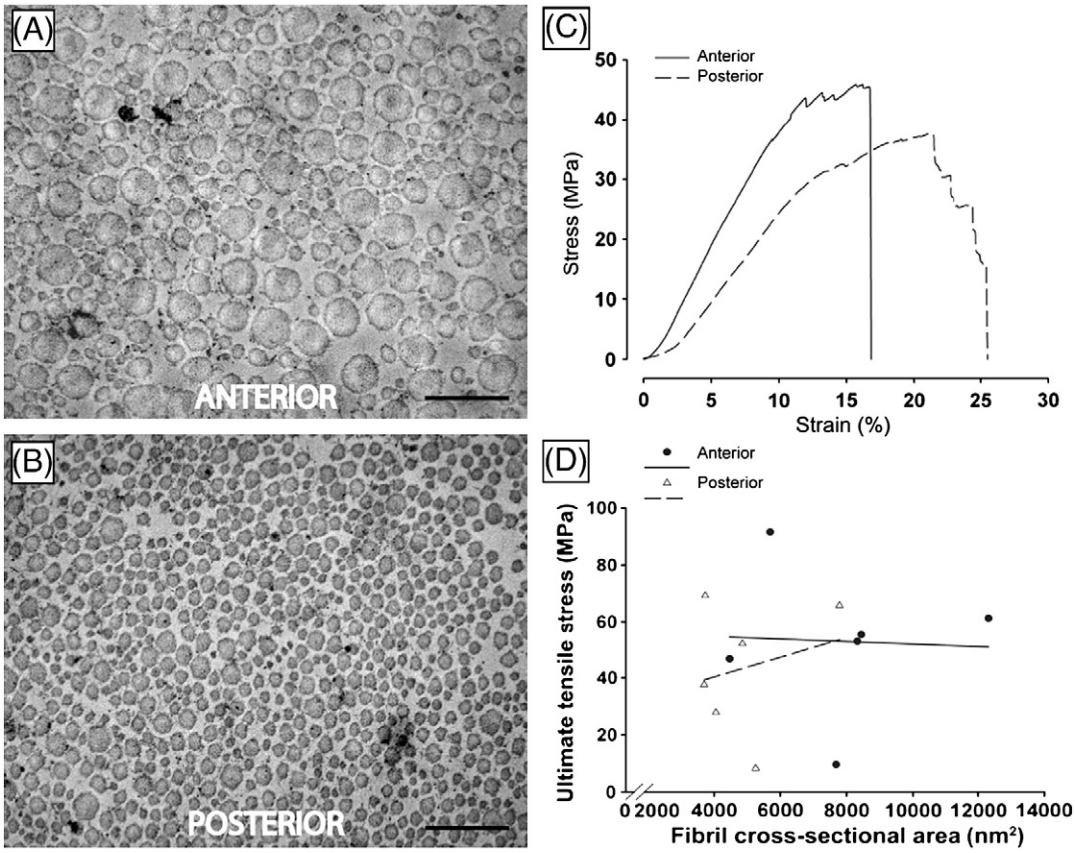

Fig. 3. Representative transmission electron microscopy images of transverse sections of collagen fascicles from the anterior (A) and posterior (B) aspect of the human patellar tendon. Posterior fascicles tended to have smaller fibril cross-sectional area. (C) Graph showing typical stress–strain properties of one anterior compared with one posterior tendon fascicle from the patellar tendon of one patient. On average, anterior fascicles were stronger and stiffer than posterior fascicles. (D) First-order linear regression plot of fibril cross-sectional area against peak stress of anterior versus posterior fascicles. There was no association between fascicle strength and fibril cross-sectional area.
Figures reproduced from Hansen et al., 2010.

regions of the stress–strain curve (Miller et al., 2012d). This suggests that re-alignment is the principal mechanism of preconditioning, providing an explanation for increased tendon strength seen after preconditioning in highly aligned tendon. Additionally, this suggests that mature tendon responds to load structurally after only a small amount of load application; this response may be related to interfibrillar communication or load transfer but the mechanism is not yet clear. However, the ability of collagen fibrils to re-orient themselves does imply some form of interfibrillar connections, whether through proteoglycan GAG chains or friction or hydrostatic forces, etc.

4.3. Collagen fibril crimp

Crimp is a periodic waveform configuration visualized within the collagen fibers and has been implicated in the mechanical behavior of collagen (Woo et al., 2000). Particularly, the flattening or disappearance of the crimp morphology has been implicated in the non-linear behavior observed in the toe-region of the stress–strain curve (Fig. 2) (Diamant et al., 1972; Atkinson et al., 1999). Further analysis of crimp morphology under scanning electron microscopy and transmission electron microscopy revealed characteristic 'knots' located within collagen fibrils. Higher magnification revealed that these knots were irregular collagen fibril segments which appeared squeezed, twisted, or bent. These knots, called 'fibrillar crimp,' corresponded to the crimp morphology visualized at the fiber level. The disappearance of crimp in collagen fiber bundles with mechanical testing as visualized by polarized light microscopy has been well documented (Jozsa and Kannus, 1997; Hansen et al., 2002), but few have been able to quantify crimp during the mechanical test. Using a custom freeze-spraying technique, our lab has captured changes in crimp frequency throughout mechanical testing. Analysis of mouse supraspinatus tendon demonstrated that the uncrimping of collagen fibers along the entirety of the tendon was confined to the toe-region (Miller et al., 2012b). Analysis of fibrillar crimp has further recognized that although fiber crimp flattens and/or disappears with tension, molecular crimp was not extinguished even within straightening of fibril bundles. This finding implies that this may be the fundamental morphology involved in tendon shock absorption (Franchi et al., 2007a).

4.4. Proteoglycans in mechanics

The role of SLRPs and GAGs in tendon's response to load has been highly debated over the past decade (Screen et al., 2005; Lujan et al., 2007; Fessel and Snedeker, 2009; Lujan et al., 2009; Fessel et al., 2012a). It has been speculated that GAGs may mechanically interconnect adjacent collagen fibrils, specifically, the GAG chains link to decorin. Relative movements of stained GAGs during mechanical relaxation tests have been quantified which led to interfibrillar force transfer through a ratchet mechanism (Cribb and Scott, 1995). However, studies with enzymatic removal of GAGs in tendon and ligament have not provided conclusive evidence to suggest this mechanism (Fessel and Snedeker, 2009; Fessel et al., 2012a). In ligament, the tensile behavior of human medial collateral ligament (MCL) was unchanged after removal of dermatan sulfate, a common GAG in ligament and tendon (Lujan et al., 2007). Similarly, rat tail tendon fascicles after incubation in chondroitinase ABC (cABC) did not have a significant effect on elastic or viscoelastic properties.

There have been a few studies to suggest that GAGs and SLRPs may play a role in static and dynamic properties (Rigozzi et al., 2009). Achilles tendons after cABC incubation did show a significant decrease in ultimate load and modulus in the midsubstance of the tendon (Fessel and Snedeker, 2009). In addition, in vitro work has shown that the addition of decorin improves mechanical properties of self-assembled collagen fibrils and simulated collagen threads (Pins et al., 1997; Kishore et al., 2011). The effect of proteoglycans and their GAG chains together has been studied through the ability to create various strains of transgenic animals. Using tail tendon fascicles from decorin knockout mice, decorin was shown to largely modulate changes in viscoelastic parameters (Elliott et al., 2003). Fascicles from decorin knockout mice showed little differences in elastic properties, but did have reduced strain rate sensitivity (Robinson et al., 2004b). Three different tendons were characterized from decorin and biglycan knockout mice to find that the loss of decorin and biglycan affected tendons differently, suggesting a role for proteoglycans that is specific to the location and/or function of the tendon (Robinson et al., 2005). Most recently, the influence of decorin on the patellar tendon mechanical properties was investigated in a dose dependent manner. This study found no differences in elastic or compressive properties but a dependence on strain rate and frequency, necessitating further exploration into the dynamic behavior of decorin and biglycan knockout tendons (Dourte et al., 2012).

4.5. Collagen cross-links

In addition to collagen and proteoglycans, the molecular cross-linking of collagen has also been speculated to play a role in tendon's response to load. Cross-linking provides structural integrity to collagen fibrils and could also help to transfer load within and between fibrils (Uzel and Buehler, 2011). This has also been demonstrated experimentally by balancing molecular slip and stretch under load (Mosler et al., 1985). Much of this research has branched off from the study of advanced glycation end-products (AGE), such as the cross-link pentosidine, which develops with aging and hyperglycemia associated with diabetes and can cause mechanical changes in tendon. Cross-links have been shown to increase with age, but this has not been correlated to changes in mechanical properties (Couppe et al., 2009). Studies investigating the mechanical properties of tendon with age in animals however have been inconclusive (Vogel, 1983; Haut et al., 1992; Dressler et al., 2002). Similarly, there have been a number of contradictory reports attempting to correlate cross-link density with tissue stiffness (Goh et al., 2008; de Oliveira et al., 2011; Fessel et al., 2012a). To date, there has not been conclusive evidence to confirm the structure–function relationship between collagen cross-linking and mechanical properties. Recent interest has focused on collagen cross-linking as a potential therapy to improve mechanical properties of healing tendon via improved repair strength (Fessel et al., 2012a, b) but more investigation into the contribution of cross-linking to tendon's response to load is warranted.

5. Developmental response to load

The response to load is altered throughout development as the structure of tendon changes. Several studies have been focused on determining how that structure changes throughout development and how those changes correspond to changes in static and dynamic mechanical properties as well as collagen fiber re-alignment and uncrimping (Ansorge et al., 2011).

5.1. Mechanical properties through development

Throughout development, cross-sectional area increases as fibrillogenesis yields longer and thicker collagen fibrils with both an increased average fibril diameter and a larger distribution of diameters. While there has not been a direct correlation between fibril diameter and mechanical properties, studies have shown that mechanical properties also increase during development (Ansorge et al., 2011; Ansorge et al., 2012a; Miller et al., 2012a). In addition, the point at which the tendon transitions from the toe region to the linear region decreases, suggesting an earlier response to the application of load. This could be due to the increasing strength of the fibrils themselves or an increase in the interconnectivity of fibers thus allowing them to communicate and transfer load more effectively. Similarly, the moduli

of both the toe and linear regions of the stress–strain curve increased, suggesting a stronger tendon (Ansorge et al., 2011; Miller et al., 2012a). However, changes between the insertion and the midsubstance of the tendon did exist at all time points, suggesting location-dependent development (Miller et al., 2012a).

5.2. Collagen re-alignment and uncrimping during development

The study of developing neonatal tendon allows analysis of the response of immature collagen to load, as opposed to mature fiber/fibril–matrix interactions. It has recently been elucidated that the developmental age of the tendon significantly influences its ability to structurally respond to load via collagen re-alignment (Miller et al., 2012a). In the midsubstance, four day old tendon was found to have prominent re-alignment within the linear region of the stress–strain curve while 10 day old tendon re-aligned within the toe-region, and 28 day old demonstrated re-alignment during preconditioning. This could suggest an earlier response to loading as the tendon develops and implicates the structural connections developing in modulating this response, in particular the maturity of collagen fibrils, proteoglycans, and collagen cross-linking. This response was not the same in the insertion site, and this work implies that the midsubstance and the insertion site of the tendon may develop at different rates. In addition, a lower linear modulus was found at the tendon insertion site than the midsubstance at all timepoints, indicating that the local structure is not the same in these two locations even at early timepoints. The developmental age of the tendon, but perhaps more importantly the structural maturity of the extracellular matrix, significantly influences its biomechanical response to load (Miller et al., 2012a).

As with re-alignment, the analysis of uncrimping characteristics of collagen fibers in response to mechanical load during development provides insight into the internal structural changes of neonatal tendon. Crimp quantification in developing tendon confirmed fiber crimp frequency as a mechanism for non-linear behavior in the toe-region of mouse supraspinatus tendon regardless of developmental age. However, between 10 and 28 day old mice, no changes in crimp frequency were found, suggesting that perhaps crimp presents at a very early age and acts as a shock absorber for the small loads until the rest of the tendon is developed. Further crimp analysis found that 28 day old tendon demonstrated small changes in crimp frequency with different levels of preconditioning (Miller et al., 2012c). This suggests that structural changes in tendon crimp morphology are affected by mechanical loading.

6. Tendon repair based on structure and function

Injuries and degenerative conditions in tendon and ligaments represent almost 50% of the musculoskeletal injuries treated in orthopaedic clinics (Schweitzer et al., 2010). Understanding the healing process and how it differs from natural tendon development could lead to better approaches to tendon repair. Tendon healing generally occurs in three overlapping stages: the inflammatory stage, the repair stage and the remodeling stage. The first stage is the recruitment of erythrocytes and neutrophils and the migration of cells to the injury in order to synthesize and deposit collagen. In the second phase, the repair phase, extracellular matrix production and growth factor expression is high. Finally, the remodeling phase begins; cellularity decreases, repair tissue becomes fibrous, collagen synthesis occurs, and collagen fibers become more aligned along the direction of loading (Sharma and Maffulli, 2005).

6.1. Alterations in healing tendon

After surgical repair of injured tendon, the 'healed' tissue is mainly disorganized scar tissue at the site of injury and limited recreation of the natural insertion site, even at 16 weeks post-repair in rats (Thomopoulos et al., 2002). Fibrils of healing tendon lose their parallel arrangement early during healing and the scar consists of mainly disorganized collagen until remodeling occurs (Ansorge et al., 2011). The mechanical properties of repaired tendons never return to uninjured levels and the complex transitional tissue characteristic of the insertion site is not recreated. There are also distinct structural alterations that occur in healing tendon and ligament. Studies investigating the scar of healing rabbit MCL showed a much weaker tissue than native tendon, marked with decreased mechanical properties even 40 weeks after injury (Frank et al., 1995). Collagen crosslink pyridinoline (PYD) density is also decreased and does not recover, even while collagen content of the tendon does return to normal levels. This could suggest a role for collagen cross-linking in modulating post-healing tendon mechanical properties. An assessment of collagen fibril diameters in healing MCL scar reveals a homogenous population of small fibrils for the first 40 weeks after injury. Some larger fibrils and heterogeneity appeared at 78 and 104 weeks but still roughly 90% of the scar was filled with small fibrils (Frank et al., 1997). In mouse Achilles tendon, the same result is found; fibril diameter size distribution consists of mainly small diameter fibrils for an extended time post-injury (Ansorge et al., 2011). The lack of recapitulation of native structure could be a factor in the reduced mechanical ability of the tissue.

There are many biological changes in scar tissue when compared with native tendon and ligament as well. Soft tissue ligament scars have decreased collagen I levels, increased collagens III and V levels and abnormal PYD levels (Nakamura et al., 2000). In addition, the levels of proteoglycans are altered in healing Achilles tendon, with increased biglycan and decorin levels after injury (Ansorge et al., 2012b). The balance between matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) is upset after injury as well, causing excessive degradation and subsequent weakening of the extracellular matrix (Garofalo et al., 2011). A recent review chronicles the changes seen in five major growth factors during tendon and ligament healing, noting that there is upregulation of many growth factors after injury and they are active at certain stages during the healing process (Molloy et al., 2003). This can be seen as evidence for a highly complex active healing process in tendons and ligaments that requires a temporal release of biological agents, not unlike the process of tendon development.

6.2. Approaches to improved tendon repair

Tissue healing and development have many similarities including cell migration and proliferation as well as extracellular matrix formation. These two highly complex processes also have some differences which researchers have been exploring to improve repair processes. One hot topic is the relationship between fetal and adult healing. Due to the weak tissue that comprises the scar in healed tendon, insight into scarless healing, which occurs naturally during fetal healing, could result in augmentation strategies to reduce scar tissue. In fetal healing, fibroblasts are present in greater numbers, collagen and elastin are more rapidly produced and granulation tissue forms more quickly (Ansorge et al., 2011). While similar temporal expressions of many growth factors occur in fetal and adult healing, there is a marked difference between the levels of transforming growth factor-beta (TGF-β) isoforms with high levels of TGF-β3 and low levels of TGF-β1 and TGF-β2 in fetal healing, resulting in no scar tissue (Galatz et al., 2006; Manning et al., 2011). Researchers have recently attempted to recreate this healing environment by altering TGF-β levels in healing adult tendon. Adding a neutralizing antibody to TGF-β1,2 to cutaneous wounds in adult rats resulted in reduced extracellular matrix deposition, reduced inflammatory cells, and increased organization of collagen deposition, which represents reduced scarring overall (Shah et al., 1995). In tendons, adding exogenous TGF-β3 using a heparin/fibrin-based delivery system to the healing tendon-to-bone insertion site lead to an

accelerated healing process and improvements in structural and material properties at later timepoints (Manning et al., 2011).

Perhaps the most direct approach to improving healing could be to try to recreate the native structure of tendon, specifically those structures believed to be directly linked with mechanical properties. With respect to this approach, there are several main structures to mimic: (1) the alignment of collagen fibers, (2) the gradient of properties such as mineralization, proteoglycan content, collagen type distribution, etc. along the tendon insertion site, (3) the level of collagen cross-linking, and (4) the natural crimp pattern of collagen fibers. Recent studies have suggested an important role for the re-alignment of collagen fibers in modulating tendon's response to load. Several groups have attempted to improve the alignment of collagen in repaired tendon using augmentation with scaffold materials (Baker et al., 2008; Xie et al., 2010; Beason et al., 2012). Fiber-aligned scaffolds can be produced that have fiber populations with similar size and distribution of fiber diameters to that of native tendon. These scaffolds direct cells seeded on them in vitro to become aligned with the axis of the fibers similar to the native alignment of tendon fibroblasts, and to then subsequently produce organized extracellular matrix along that axis (Baker et al., 2008; Moffat et al., 2009). In vivo, these scaffolds alone have not been able to improve mechanical properties of a repaired tendon even 8 weeks after implantation, suggesting perhaps fiber alignment is not enough to direct proper tissue deposition (Beason et al., 2012).

To mimic the gradient of properties at the tendon insertion site, biphasic aligned polymer scaffolds have been produced (Moffat et al., 2011). The scaffolds have a non-mineralized and a mineralized region that is designed to mimic the insertion site gradient of mineralization. No current mechanical data is published for these scaffolds, but in vitro work and histology shows promising results for recreation of the tendon insertion site. Chondrocyte viability and proliferation as well as synthesis of a GAG and collagen-rich matrix were both supported in this matrix. In addition, there was deposition of a continuous non-calcified and calcified fibrocartilage when implanted over a repaired supraspinatus tendon for 5 weeks. In addition to mimicking the mineralization of tendon, several studies have started to focus on the proteoglycan content of tendon, specifically decorin. Since decorin is responsible for limiting the lateral growth of collagen fibrils during development, upregulation of decorin post-injury could be responsible for the lack of large collagen fibrils and a heterogeneous distribution in healing tendon (Zhang et al., 2006). Recent studies have investigated decorin antisense gene therapy as one way to reduce the levels of decorin following injury (Hart et al., 2000; Nakamura et al., 2000; Hosaka et al., 2005). Decorin suppression in vitro in tendinocytes from rabbit Achilles tendon prevented decorin deposition in scar tissue but also suppressed TGF-β1 production (Hosaka et al., 2005). Down-regulation of decorin using this method in rabbit ligament resulted in development of larger collagen fibrils in early scar as well as improved failure strength at 6 weeks, although it's important to note that this therapy also affects other genes making drawing conclusions difficult (Hart et al., 2000; Nakamura et al., 2000).

Finally, there has been some effort to improve the structure and function of repaired tendon by studying native collagen cross-links. While it is extensively studied, the evidence of correlation with increased collagen cross-links and mechanical properties has been rather weak (Fessel et al., 2012a). More investigation into the role of collagen cross-links in modulating load in tendon is necessary to draw conclusions into how they can improve repaired tissues. However, in vitro work adding exogenous cross-linkers to a biopsy-punched tendon showed that cross-link treatment resulted in recovery of the loss of mechanical integrity indicated by reduced tissue strain and increased modulus (Fessel et al., 2012b). Assessment in an in vivo model is warranted given this promising result. More investigation is also warranted into the changes in collagen crimp seen after injury. In tendons allowed to retract after injury, increased fiber crimping and disorganization was found, suggesting a role for continuous mechanical loading in maintaining appropriate fiber crimp (Farshad et al., 2011). Combined with decreased in crimp frequency found throughout development (Miller et al., 2012c), collagen crimp may be an important mechanism of tendon's response to load and more research into this phenomenon is necessary.

7. Conclusion

Development of tendon is a complex process that leads to a unique hierarchical structure that exhibits nonlinear, anisotropic, and viscoelastic mechanical behavior. The structure and composition of tendon is directly related to its function via mechanisms of collagen fiber re-alignment and uncrimping. Healing tendons do not exhibit the same structure as native tendon since adult healing is a reparative, and not regenerative, process. In addition, these healed tendons do not exhibit the same mechanical behavior as native tendon, suggesting the important relationship between structure and function. While some researchers have begun to explore this relationship in order to improve the healing of tendon with promising results, much more investigation is necessary.

References

Ansorge, H.L., Meng, X., Zhang, G., Veit, G., Sun, M., Klement, J.F., Beason, D.P., Soslowsky, L.J., Koch, M., Birk, D.E., 2009. Type XIV collagen regulates fibrillogenesis: premature collagen fibril growth and tissue dysfunction in null mice. J. Biol. Chem. 284, 8427–8438.

Ansorge, H., Adams, S., Birk, D., Soslowsky, L., 2011. Mechanical, compositional, and structural properties of the post-natal mouse Achilles tendon. Ann. Biomed. Eng. 39, 1904–1913.

Ansorge, H.L., Adams, S., Jawad, A.F., Birk, D.E., Soslowsky, L.J., 2012a. Mechanical property changes during neonatal development and healing using a multiple regression model. J. Biomech. 45, 1288–1292.

Ansorge, H.L., Hsu, J.E., Edelstein, L., Adams, S., Birk, D.E., Soslowsky, L.J., 2012b. Recapitulation of the Achilles tendon mechanical properties during neonatal development: a study of differential healing during two stages of development in a mouse model. J. Orthop. Res. 30, 448–456.

Atkinson, T.S., Ewers, B.J., Haut, R.C., 1999. The tensile and stress relaxation responses of human patellar tendon varies with specimen cross-sectional area. J. Biomech. 32, 907–914.

Baker, B.M., Gee, A.O., Metter, R.B., Nathan, A.S., Marklein, R.A., Burdick, J.A., Mauck, R.L., 2008. The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. Biomaterials 29, 2348–2358.

Beason, D.P., Connizzo, B.K., Dourte, L.M., Mauck, R.L., Soslowsky, L.J., Steinberg, D.R., Bernstein, J., 2012. Fiber-aligned polymer scaffolds for rotator cuff repair in a rat model. J. Should. Elb. Surg. 21, 245–250.

Benjamin, M., Ralphs, J.R., 2000. The cell and developmental biology of tendons and ligaments. Int. Rev. Cytol. 196, 85–130.

Benjamin, M., Evans, E.J., Copp, L., 1986. The histology of tendon attachments to bone in man. J. Anat. 149, 89–100.

Benjamin, M., Kumai, T., Milz, S., Boszczyk, B.M., Boszczyk, A.A., Ralphs, J.R., 2002. The skeletal attachment of tendons—tendon "entheses". Comp. Biochem. Physiol. A Mol. Integr. Physiol. 133, 931–945.

Birk, D.E., 2001. Type V collagen: heterotypic type I/V collagen interactions in the regulation of fibril assembly. Micron 32, 223–237.

Birk, D.E., Mayne, R., 1997. Localization of collagen types I, III and V during tendon development. Changes in collagen types I and III are correlated with changes in fibril diameter. Eur. J. Cell Biol. 72, 352–361.

Birk, D.E., Silver, F.H., Trelstad, R.L., 1991. Matrix assembly. In: Hay, E.D. (Ed.), Cell Biology of Extracellular Matrix. Plenum Press, New York.

Birk, D.E., Nurminskaya, M.V., Zycband, E.I., 1995. Collagen fibrillogenesis in situ: fibril segments undergo post-depositional modifications resulting in linear and lateral growth during matrix development. Dev. Dyn. 202, 229–243.

Birk, D.E., Zycband, E.I., Woodruff, S., Winkelmann, D.A., Trelstad, R.L., 1997. Collagen fibrillogenesis in situ: fibril segments become long fibrils as the developing tendon matures. Dev. Dyn. 208, 291–298.

Brent, A.E., Braun, T., Tabin, C.J., 2005. Genetic analysis of interactions between the somitic muscle, cartilage and tendon cell lineages during mouse development. Development 132, 515–528.

Christiansen, D.L., Huang, E.K., 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, 409–420.

Couppe, C., Hansen, P., Kongsgaard, M., Kovanen, V., Suetta, C., Aagaard, P., Kjaer, M., Magnusson, S.P., 2009. Mechanical properties and collagen cross-linking of the patellar tendon in old and young men. J. Appl. Physiol. 107, 880–886.

Cribb, A.M., Scott, J.E., 1995. Tendon response to tensile stress: an ultrastructural investigation of collagen:proteoglycan interactions in stressed tendon. J. Anat. 187 (Pt 2), 423–428.

de Oliveira, R.R., de Lira, K.D., Silveira, P.V., Coutinho, M.P., Medeiros, M.N., Teixeira, M.F., de Moraes, S.R., 2011. Mechanical properties of achilles tendon in rats induced to experimental diabetes. Ann. Biomed. Eng. 39, 1528–1534.
Deries, M., Schweitzer, R., Duxson, M.J., 2010. Developmental fate of the mammalian myotome. Dev. Dyn. 239, 2898–2910.
Derwin, K.A., Soslowsky, L.J., 1999. A quantitative investigation of structure–function relationships in a tendon fascicle model. J. Biomech. Eng. 121, 598–604.
Derwin, K.A., Soslowsky, L.J., Kimura, J.H., Plaas, A.H., 2001. Proteoglycans and glycosaminoglycan fine structure in the mouse tail tendon fascicle. J. Orthop. Res. 19, 269–277.
Diamant, J., Keller, A., Baer, E., Litt, M., Arridge, R.G., 1972. Collagen; ultrastructure and its relation to mechanical properties as a function of ageing. Proc. R. Soc. Lond. B Biol. Sci. 180, 293–315.
Dourte, L.M., Pathmanathan, L., Jawad, A.F., Iozzo, R.V., Mienaltowski, M.J., Birk, D.E., Soslowsky, L.J., 2012. Influence of decorin on the mechanical, compositional, and structural properties of the mouse patellar tendon. J. Biomech. Eng. 134, 031005.
Dressler, M.R., Butler, D.L., Wenstrup, R., Awad, H.A., Smith, F., Boivin, G.P., 2002. A potential mechanism for age-related declines in patellar tendon biomechanics. J. Orthop. Res. 20, 1315–1322.
Einhorn, T.A., O'Keefe, R.J., Buckwalter, J.A., American Academy of Orthopaedic Surgeons, 2007. Orthopaedic Basic Science: Foundations of Clinical Practice. American Academy of Orthopaedic Surgeons, Rosemont, IL.
Elliott, D.M., Robinson, P.S., Gimbel, J.A., Sarver, J.J., Abboud, J.A., Iozzo, R.V., Soslowsky, L.J., 2003. Effect of altered matrix proteins on quasilinear viscoelastic properties in transgenic mouse tail tendons. Ann. Biomed. Eng. 31, 599–605.
Farshad, M., Gerber, C., Snedeker, J.G., Frauenfelder, T., Meyer, D.C., 2011. Structure of retracted tendons after staged repair following continuous traction. Knee Surg. Sports Traumatol. Arthrosc. 19, 2131–2137.
Fessel, G., Snedeker, J.G., 2009. Evidence against proteoglycan mediated collagen fibril load transmission and dynamic viscoelasticity in tendon. Matrix Biol. 28, 503–510.
Fessel, G., Gerber, C., Snedeker, J.G., 2012a. Potential of collagen cross-linking therapies to mediate tendon mechanical properties. J. Should. Elb. Surg. 21, 209–217.
Fessel, G., Wernli, J., Li, Y., Gerber, C., Snedeker, J.G., 2012b. Exogenous collagen cross-linking recovers tendon functional integrity in an experimental model of partial tear. J. Orthop. Res. 30, 973–981.
Franchi, M., Fini, M., Quaranta, M., De Pasquale, V., Raspanti, M., Giavaresi, G., Ottani, V., Ruggeri, A., 2007a. Crimp morphology in relaxed and stretched rat Achilles tendon. J. Anat. 210, 1–7.
Franchi, M., Trire, A., Quaranta, M., Orsini, E., Ottani, V., 2007b. Collagen structure of tendon relates to function. Sci. World J. 7, 404–420.
Frank, C., McDonald, D., Wilson, J., Eyre, D., Shrive, N., 1995. Rabbit medial collateral ligament scar weakness is associated with decreased collagen pyridinoline crosslink density. J. Orthop. Res. 13, 157–165.
Frank, C., McDonald, D., Shrive, N., 1997. Collagen fibril diameters in the rabbit medial collateral ligament scar: a longer term assessment. Connect. Tissue Res. 36, 261–269.
Galatz, L.M., Sandell, L.J., Rothermich, S.Y., Das, R., Mastny, A., Havlioglu, N., Silva, M.J., Thomopoulos, S., 2006. Characteristics of the rat supraspinatus tendon during tendon-to-bone healing after acute injury. J. Orthop. Res. 24, 541–550.
Garofalo, R., Cesari, E., Vinci, E., Castagna, A., 2011. Role of metalloproteinases in rotator cuff tear. Sports Med. Arthrosc. 19, 207–212.
Gautieri, A., Vesentini, S., Redaelli, A., Buehler, M.J., 2011. Hierarchical structure and nanomechanics of collagen microfibrils from the atomistic scale up. Nano Lett. 11, 757–766.
Gautieri, A., Vesentini, S., Redaelli, A., Buehler, M.J., 2012. Viscoelastic properties of model segments of collagen molecules. Matrix Biol. 31, 141–149.
Genin, G.M., Kent, A., Birman, V., Wopenka, B., Pasteris, J.D., Marquez, P.J., Thomopoulos, S., 2009. Functional grading of mineral and collagen in the attachment of tendon to bone. Biophys. J. 97, 976–985.
Goh, K.L., Holmes, D.F., Lu, H.Y., Richardson, S., Kadler, K.E., Purslow, P.P., Wess, T.J., 2008. Ageing changes in the tensile properties of tendons: influence of collagen fibril volume fraction. J. Biomech. Eng. 130, 021011.
Hansen, K.A., Weiss, J.A., Barton, J.K., 2002. Recruitment of tendon crimp with applied tensile strain. J. Biomech. Eng. 124, 72–77.
Hansen, P., Haraldsson, B.T., Aagaard, P., Kovanen, V., Avery, N.C., Qvortrup, K., Larsen, J.O., Krogsgaard, M., Kjaer, M., Peter Magnusson, S., 2010. Lower strength of the human posterior patellar tendon seems unrelated to mature collagen cross-linking and fibril morphology. J. Appl. Physiol. 108, 47–52.
Hart, D.A., Nakamura, N., Marchuk, L., Hiraoka, H., Boorman, R., Kaneda, Y., Shrive, N.G., Frank, C.B., 2000. Complexity of determining cause and effect in vivo after antisense gene therapy. Clin. Orthop. Relat. Res. S242–S251.
Haut, R.C., Lancaster, R.L., DeCamp, C.E., 1992. Mechanical properties of the canine patellar tendon: some correlations with age and the content of collagen. J. Biomech. 25, 163–173.
Heim, A.J., Koob, T.J., Matthews, W.G., 2007. Low strain nanomechanics of collagen fibrils. Biomacromolecules 8, 3298–3301.
Hosaka, Y., Kirisawa, R., Mafune, N., Takehana, K., 2005. Downregulation of decorin and transforming growth factor-beta1 by decorin gene suppression in tendinocytes. Connect. Tissue Res. 46, 18–26.
Iozzo, R.V., Murdoch, A.D., 1996. Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. FASEB J. 10, 598–614.
Jozsa, L., Kannus, P., 1997. Histopathological findings in spontaneous tendon ruptures. Scand. J. Med. Sci. Sports 7, 113–118.
Kalamajski, S., Oldberg, A., 2010. The role of small leucine-rich proteoglycans in collagen fibrillogenesis. Matrix Biol. 29, 248–253.
Kalson, N.S., Holmes, D.F., Herchenhan, A., Lu, Y., Starborg, T., Kadler, K.E., 2011. Slow stretching that mimics embryonic growth rate stimulates structural and mechanical development of tendon-like tissue in vitro. Dev. Dyn. 240, 2520–2528.
Killian, M.L., Cavinatto, L., Galatz, L.M., Thomopoulos, S., 2012. The role of mechanobiology in tendon healing. J. Should. Elb. Surg. 21, 228–237.
Kishore, V., Paderi, J.E., Akkus, A., Smith, K.M., Balachandran, D., Beaudoin, S., Panitch, A., Akkus, O., 2011. Incorporation of a decorin biomimetic enhances the mechanical properties of electrochemically aligned collagen threads. Acta Biomater. 7, 2428–2436.
Lake, S.P., Miller, K.S., Elliott, D.M., Soslowsky, L.J., 2009. Effect of fiber distribution and realignment on the nonlinear and inhomogeneous mechanical properties of human supraspinatus tendon under longitudinal tensile loading. J. Orthop. Res. 27, 1596–1602.
Lake, S.P., Miller, K.S., Elliott, D.M., Soslowsky, L.J., 2010. Tensile properties and fiber alignment of human supraspinatus tendon in the transverse direction demonstrate inhomogeneity, nonlinearity, and regional isotropy. J. Biomech. 43, 727–732.
Liu, W., Watson, S.S., Lan, Y., Keene, D.R., Ovitt, C.E., Liu, H., Schweitzer, R., Jiang, R., 2010. The atypical homeodomain transcription factor Mohawk controls tendon morphogenesis. Mol. Cell. Biol. 30, 4797–4807.
Lujan, T.J., Underwood, C.J., Henninger, H.B., Thompson, B.M., 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, 894–903.
Lujan, T.J., Underwood, C.J., Jacobs, N.T., Weiss, J.A., 2009. Contribution of glycosaminoglycans to viscoelastic tensile behavior of human ligament. J. Appl. Physiol. 106, 423–431.
Maeda, T., Sakabe, T., Sunaga, A., Sakai, K., Rivera, A.L., Keene, D.R., Sasaki, T., Stavnezer, E., Iannotti, J., Schweitzer, R., Ilic, D., Baskaran, H., Sakai, T., 2011. Conversion of mechanical force into TGF-beta-mediated biochemical signals. Curr. Biol. 21, 933–941.
Manning, C.N., Kim, H.M., Sakiyama-Elbert, S., Galatz, L.M., Havlioglu, N., Thomopoulos, S., 2011. Sustained delivery of transforming growth factor beta three enhances tendon-to-bone healing in a rat model. J. Orthop. Res. 29, 1099–1105.
McNeilly, C.M., Banes, A.J., Benjamin, M., Ralphs, J.R., 1996. Tendon cells in vivo form a three dimensional network of cell processes linked by gap junctions. J. Anat. 189 (Pt 3), 593–600.
Miller, K., Connizzo, B., Soslowsky, L., 2012a. Collagen fiber re-alignment in a neonatal developmental mouse supraspinatus tendon model. Ann. Biomed. Eng. 40, 1102–1110.
Miller, K.S., Connizzo, B.K., Feeney, E., Soslowsky, L.J., 2012b. Characterizing local collagen fiber re-alignment and crimp behavior throughout mechanical testing in a mature mouse supraspinatus tendon model. J. Biomech. 45, 2061–2065.
Miller, K.S., Connizzo, B.K., Feeney, E., Tucker, J.J., Soslowsky, L.J., 2012c. Examining differences in local collagen fiber crimp frequency throughout mechanical testing in a developmental mouse supraspinatus tendon model. J. Biomech. Eng. 134 (4), 041004.
Miller, K.S., Edelstein, L., Connizzo, B.K., Soslowsky, L.J., 2012d. Effect of preconditioning and stress relaxation on local collagen fiber re-alignment: inhomogeneous properties of rat supraspinatus tendon. J. Biomech. Eng. 134 (3), 031007.
Moffat, K.L., Kwei, A.S., Spalazzi, J.P., Doty, S.B., Levine, W.N., Lu, H.H., 2009. Novel nanofiber-based scaffold for rotator cuff repair and augmentation. Tissue Eng. A 15, 115–126.
Moffat, K., Zhang, C., Greco, S., Boushell, M., Guo, X., Doty, S., Soslowsky, L., Levine, W., Lu, H., 2011. In vitro and in vivo evaluation of a bi-phasic nanofiber scaffold for integrative rotator cuff repair. Trans. Orthop. Res. Soc. 36, 482.
Molloy, T., Wang, Y., Murrell, G.A.C., 2003. The roles of growth factors in tendon and ligament healing. Sports Med. 33, 381–394.
Mosler, E., Folkhard, W., Knorzer, E., Nemetschek-Gansler, H., Nemetschek, T., Koch, M.H., 1985. Stress-induced molecular rearrangement in tendon collagen. J. Mol. Biol. 182, 589–596.
Murchison, N.D., Price, B.A., Conner, D.A., Keene, D.R., Olson, E.N., Tabin, C.J., Schweitzer, R., 2007. Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons. Development 134, 2697–2708.
Nakamura, N., Hart, D.A., Boorman, R.S., Kaneda, Y., Shrive, N.G., Marchuk, L.L., Shino, K., Ochi, T., Frank, C.B., 2000. Decorin antisense gene therapy improves functional healing of early rabbit ligament scar with enhanced collagen fibrillogenesis in vivo. J. Orthop. Res. 18, 517–523.
Parry, D.A., 1988. The molecular and fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue. Biophys. Chem. 29, 195–209.
Pins, G.D., Christiansen, D.L., Patel, R., Silver, F.H., 1997. Self-assembly of collagen fibers. Influence of fibrillar alignment and decorin on mechanical properties. Biophys. J. 73, 2164–2172.
Provenzano, P.P., Vanderby Jr., R., 2006. Collagen fibril morphology and organization: implications for force transmission in ligament and tendon. Matrix Biol. 25, 71–84.
Pryce, B.A., Watson, S.S., Murchison, N.D., Staverosky, J.A., Dunker, N., Schweitzer, R., 2009. Recruitment and maintenance of tendon progenitors by TGFbeta signaling are essential for tendon formation. Development 136, 1351–1361.
Quinn, K.P., Winkelstein, B.A., 2011. Preconditioning is correlated with altered collagen fiber alignment in ligament. J. Biomech. Eng. 133, 064506.
Rigozzi, S., Muller, R., Snedeker, J.G., 2009. Local strain measurement reveals a varied regional dependence of tensile tendon mechanics on glycosaminoglycan content. J. Biomech. 42, 1547–1552.
Rigozzi, S., Muller, R., Snedeker, J.G., 2010. Collagen fibril morphology and mechanical properties of the Achilles tendon in two inbred mouse strains. J. Anat. 216, 724–731.
Robinson, P.S., Lin, T.W., Jawad, A.F., Iozzo, R.V., Soslowsky, L.J., 2004a. Investigating tendon fascicle structure–function relationships in a transgenic-age mouse model using multiple regression models. Ann. Biomed. Eng. 32, 924–931.

Robinson, P.S., Lin, T.W., Reynolds, P.R., Derwin, K.A., Iozzo, R.V., Soslowsky, L.J., 2004b. Strain-rate sensitive mechanical properties of tendon fascicles from mice with genetically engineered alterations in collagen and decorin. J. Biomech. Eng. 126, 252–257.
Robinson, P.S., Huang, T.-F., Kazam, E., Iozzo, R.V., Birk, D.E., Soslowsky, L.J., 2005. Influence of decorin and biglycan on mechanical properties of multiple tendons in knockout mice. J. Biomech. Eng. 127, 181–185.
Schweitzer, R., Zelzer, E., Volk, T., 2010. Connecting muscles to tendons: tendons and musculoskeletal development in flies and vertebrates. Development 137, 2807–2817.
Scott, J.E., Orford, C.R., Hughes, E.W., 1981. Proteoglycan–collagen arrangements in developing rat tail tendon. An electron microscopical and biochemical investigation. Biochem. J. 195, 573–581.
Screen, H.R., Shelton, J.C., Chhaya, V.H., Kayser, M.V., Bader, D.L., Lee, D.A., 2005. The influence of noncollagenous matrix components on the micromechanical environment of tendon fascicles. Ann. Biomed. Eng. 33, 1090–1099.
Segev, F., Heon, E., Cole, W.G., Wenstrup, R.J., Young, F., Slomovic, A.R., Rootman, D.S., Whitaker-Menezes, D., Chervoneva, I., Birk, D.E., 2006. Structural abnormalities of the cornea and lid resulting from collagen V mutations. Invest. Ophthalmol. Vis. Sci. 47, 565–573.
Seidler, D.G., Schaefer, L., Robenek, H., Iozzo, R.V., Kresse, H., Schonherr, E., 2005. A physiologic three-dimensional cell culture system to investigate the role of decorin in matrix organisation and cell survival. Biochem. Biophys. Res. Commun. 332, 1162–1170.
Shah, M., Foreman, D.M., Ferguson, M.W., 1995. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J. Cell Sci. 108 (Pt 3), 985–1002.
Sharma, P., Maffulli, N., 2005. Basic biology of tendon injury and healing. Surgeon 3, 309–316.
Shirachi, I., Gotoh, M., Mitsui, Y., Yamada, T., Nakama, K., Kojima, K., Okawa, T., Higuchi, F., Nagata, K., 2011. Collagen production at the edge of ruptured rotator cuff tendon is correlated with postoperative cuff integrity. Arthroscopy 27, 1173–1179.
Silver, F.H., Ebrahimi, A., Snowhill, P.B., 2002. Viscoelastic properties of self-assembled type I collagen fibers: molecular basis of elastic and viscous behaviors. Connect. Tissue Res. 43, 569–580.
Svensson, L., Aszodi, A., Reinholt, F.P., Fassler, R., Heinegard, D., Oldberg, A., 1999. Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. J. Biol. Chem. 274, 9636–9647.
Svensson, R.B., Hassenkam, T., Hansen, P., Peter Magnusson, S., 2010. Viscoelastic behavior of discrete human collagen fibrils. J. Mech. Behav. Biomed. Mater. 3, 112–115.
Sverdlik, A., Lanir, Y., 2002. Time-dependent mechanical behavior of sheep digital tendons, including the effects of preconditioning. J. Biomech. Eng. 124, 78–84.
Thomopoulos, S., Hattersley, G., Rosen, V., Mertens, M., Galatz, L., Williams, G.R., Soslowsky, L.J., 2002. The localized expression of extracellular matrix components in healing tendon insertion sites: an in situ hybridization study. J. Orthop. Res. 20, 454–463.
Thomopoulos, S., Williams, G.R., Gimbel, J.A., Favata, M., Soslowsky, L.J., 2003. Variation of biomechanical, structural, and compositional properties along the tendon to bone insertion site. J. Orthop. Res. 21, 413–419.
Thomopoulos, S., Marquez, J.P., Weinberger, B., Birman, V., Genin, G.M., 2006. Collagen fiber orientation at the tendon to bone insertion and its influence on stress concentrations. J. Biomech. 39, 1842–1851.
Thomopoulos, S., Genin, G.M., Galatz, L.M., 2010. The development and morphogenesis of the tendon-to-bone insertion — what development can teach us about healing. J. Musculoskelet. Neuronal. Interact. 10, 35–45.
Tozer, S., Duprez, D., 2005. Tendon and ligament: development, repair and disease. Birth Defects Res. C Embryo Today 75, 226–236.
Uzel, S.G., Buehler, M.J., 2011. Molecular structure, mechanical behavior and failure mechanism of the C-terminal cross-link domain in type I collagen. J. Mech. Behav. Biomed. Mater. 4, 153–161.
van der Rijt, J.A., van der Werf, K.O., Bennink, M.L., Dijkstra, P.J., Feijen, J., 2006. Micromechanical testing of individual collagen fibrils. Macromol. Biosci. 6, 697–702.
Veres, S.P., Lee, J.M., 2012. Designed to fail: a novel mode of collagen fibril disruption and its relevance to tissue toughness. Biophys. J. 102, 2876–2884.
Vogel, H.G., 1983. Age dependence of mechanical properties of rat tail tendons (hysteresis experiments). Aktuelle Gerontol. 13, 22–27.
Voleti, P.B., Buckley, M.R., Soslowsky, L.J., 2012. Tendon healing: repair and regeneration. Annu. Rev. Biomed. Eng. 14, 47–71.
Wenstrup, R.J., Florer, J.B., Brunskill, E.W., Bell, S.M., Chervoneva, I., Birk, D.E., 2004. Type V collagen controls the initiation of collagen fibril assembly. J. Biol. Chem. 279, 53331–53337.
Wenstrup, R.J., Smith, S.M., Florer, J.B., Zhang, G., Beason, D.P., Seegmiller, R.E., Soslowsky, L.J., Birk, D.E., 2011. Regulation of collagen fibril nucleation and initial fibril assembly involves coordinate interactions with collagens V and XI in developing tendon. J. Biol. Chem. 286, 20455–20465.
Woo, S.L., Debski, R.E., Zeminski, J., Abramowitch, S.D., Saw, S.S., Fenwick, J.A., 2000. Injury and repair of ligaments and tendons. Annu. Rev. Biomed. Eng. 2, 83–118.
Woo, S.L., Thay, Q.L., Abramowitch, S.D., Gilbert, T.W., 2005. Structure and function of ligaments and tendons, Basic orthopaedic Biomechanics & Mechano-biology, 3rd ed. Lippincott, Williams & Wilkins, Philadelphia [u.a.], pp. 301–342.
Wopenka, B., Kent, A., Pasteris, J.D., Yoon, Y., Thomopoulos, S., 2008. The tendon-to-bone transition of the rotator cuff: a preliminary Raman spectroscopic study documenting the gradual mineralization across the insertion in rat tissue samples. Appl. Spectrosc. 62, 1285–1294.
Xie, J., Li, X., Lipner, J., Manning, C.N., Schwartz, A.G., Thomopoulos, S., Xia, Y., 2010. "Aligned-to-random" nanofiber scaffolds for mimicking the structure of the tendon-to-bone insertion site. Nanoscale 2, 923–926.
Zhang, G., Young, B.B., Ezura, Y., Favata, M., Soslowsky, L.J., Chakravarti, S., Birk, D.E., 2005. Development of tendon structure and function: regulation of collagen fibrillogenesis. J. Musculoskelet. Neuronal. Interact. 5, 5–21.
Zhang, G., Ezura, Y., Chervoneva, I., Robinson, P.S., Beason, D.P., Carine, E.T., Soslowsky, L.J., Iozzo, R.V., Birk, D.E., 2006. Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. J. Cell. Biochem. 98, 1436–1449.