



REVIEW ARTICLE

The role of the non-collagenous matrix in tendon function

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SUMMARY

Tendon consists of highly ordered type I collagen molecules that are grouped together to form subunits of increasing diameter. At each hierarchical level, the type I collagen is interspersed with a predominantly non-collagenous matrix (NCM) (*Connect. Tissue Res.*, 6, 1978, 11). Whilst many studies have investigated the structure, organization and function of the collagenous matrix within tendon, relatively few have studied the non-collagenous components. However, there is a growing body of research suggesting the NCM plays an important role within tendon; adaptations to this matrix may confer the specific properties required by tendons with different functions. Furthermore, age-related alterations to non-collagenous proteins have been identified, which may affect tendon resistance to injury. This review focuses on the NCM within the tensional region of developing and mature tendon, discussing the current knowledge and identifying areas that require further study to fully understand structure–function relationships within tendon. This information will aid in the development of appropriate techniques for tendon injury prevention and treatment.

Keywords

ageing, glycoprotein, interfascicular matrix, proteoglycan, structure-function

Tendon composition

Tendon matrix is predominantly composed of collagen, which makes up 60–85% of the tendon dry weight (Kjaer 2004). Approximately 95% of this is type I collagen, with small amounts of collagen types III, V, XII and XIV (Riley *et al.* 1994b; Birch *et al.* 1999; Riley 2004; Banos *et al.* 2008). The collagen-rich, hierarchically arranged structure results in a tissue with high tensile strength (Figure 1). Each level of the hierarchy is interspersed with a small amount of non-collagenous matrix (NCM) (Kastelic *et al.* 1978). The role of collagen within tendon is well characterized and has been the subject of many reviews (Kannus 2000; Silver *et al.* 2003; Franchi *et al.* 2008; Starborg *et al.* 2008; Kjaer *et al.* 2009) and as such is not the main focus of this article. By contrast, the NCM is less well defined. This matrix is primarily made up of glycoproteins (Yoon & Halper 2005), which comprise a protein core covalently linked to a carbo-

hydrate, ranging in size from a monosaccharide to large polysaccharide chains. Glycoproteins include the proteoglycans and other molecules such as collagen oligomeric matrix protein (COMP), lubricin and tenascin-C. The NCM also includes the fibrous protein elastin that forms part of elastic fibre.

Proteoglycans and glycoproteins in tendon

Proteoglycans are the most abundant class of glycoproteins within tendon, consisting of one or several (often many) polysaccharide chains attached via O-linkage to a core protein. Each polysaccharide chain consists of one branch of repeating disaccharide units, which can be over 100 units long, and may be sulphated in various positions and quantities. These chains are commonly referred to as glycosaminoglycan (GAG) side chains (Sharon 1986). The majority of the proteoglycans found within tendon are the small

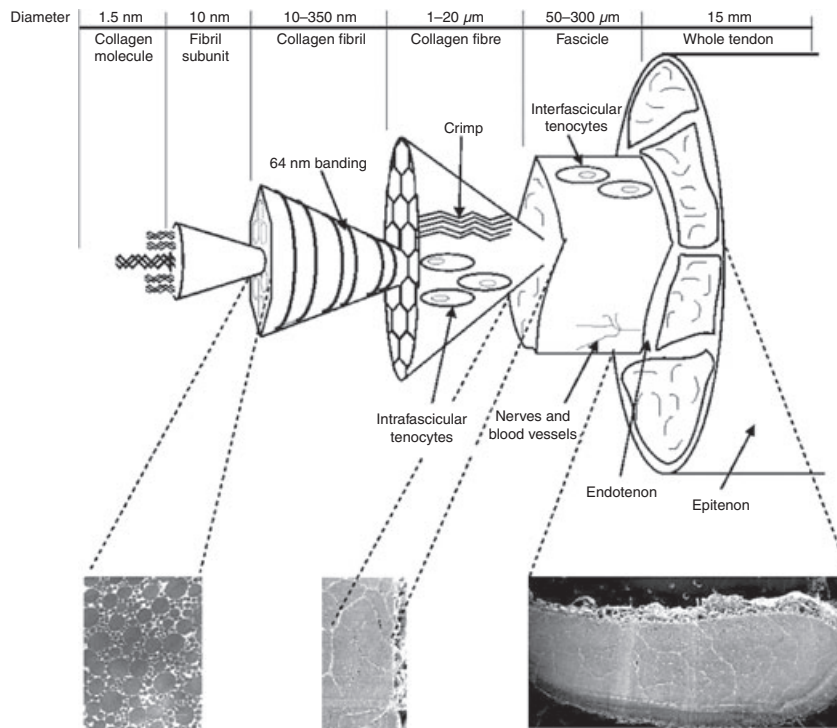


Figure 1 Schematic showing the hierarchical structure of tendon, in which collagen molecules assemble to form subunits of increasing diameter. At each level, the collagen is interspersed with a small amount of non-collagenous matrix. Adapted from Thorpe *et al.* (2010a) with permission from Wiley.

leucine-rich proteoglycans (SLRPs), with smaller amounts of the large proteoglycans aggrecan and versican (Rees *et al.* 2000; Yoon & Halper 2005). Decorin is the most abundant SLRP, accounting for approximately 80% of the total proteoglycan content (Samiric *et al.* 2004), with lower levels of biglycan, fibromodulin and lumican. Other glycoproteins identified within tendon include COMP (Smith *et al.* 1997), lubricin (Rees *et al.* 2002), tenascin-C (Riley *et al.* 1996) and tenomodulin (Brandau *et al.* 2001), whilst components of the elastic fibre are also present in varying quantities (Silver *et al.* 2003; Korol *et al.* 2007).

Whilst these non-collagenous proteins are known to be present within tendon, there is relatively little understanding of the precise location or function of specific proteins within the tendon matrix, although it has been established that the profile of this matrix differs between compressive and tensile regions within tendon. Regions that experience compressive loads, for example, where the tendon wraps round a joint, have a relative abundance of non-collagenous proteins. It is accepted that the high concentration of proteins such as aggrecan in this region increases the water content, thereby providing increased stiffness and resistance to compression (Rees *et al.* 2000; Jones & Riley 2005; Yoon & Halper 2005). By contrast, the tensile region of tendon has a relatively sparse NCM, with the role of specific proteins in this region yet to be established.

In addition to differences in NCM composition between compressive and tensile regions, the NCM varies between hierarchical levels. It is thought that there is little or no

NCM at the lowest levels of the tendon hierarchy (i.e. between collagen molecules and subfibrils), and there is evidence to suggest that the NCM becomes more complex as the collagen subunits become larger. The proteoglycans decorin and biglycan are known to be present between fibrils, fibres and fascicles (Scott 1996; Kim *et al.* 2010), whereas COMP is thought to be localized only between fibrils and fibres (Sodersten *et al.* 2005, 2013). By contrast, components such as lubricin and elastin have only been identified between fascicles (Sun *et al.* 2006; Korol *et al.* 2007). The distribution of other NCM components within tendon remains to be fully determined.

Further, in addition to spatial variations, the NCM differs between tendon types, with greater content of non-collagenous proteins in tendons that experience high strains (Smith *et al.* 2002a; Batson *et al.* 2003; Jarvinen *et al.* 2003).

Finally, there are also temporal differences in non-collagenous protein content, with changes occurring to this matrix during development, maturation and ageing. Indeed, recent research suggests distinct roles for individual non-collagenous proteins in developing and mature tendons.

Distribution of non-collagenous matrix throughout tendon hierarchy

Interfibrillar matrix

The proteins comprising the interfibrillar matrix are mainly the SLRPs, which are able to bind to collagen fibrils at

specific sites. These proteoglycans consist of a horseshoe-shaped core protein, which is able to bind non-covalently to a single collagen triple helix at a specific amino acid sequence in the gap region (D-band) of the fibril, such that molecules bind approximately every 68 nm along the collagen fibril (Scott 1996; Weber *et al.* 1996; Sweeney *et al.* 2008). Attached to the core protein is one or several GAG side chains, depending on the type of proteoglycan. Decorin has a single chondroitin or dermatan sulphate side chain, which binds to one edge of the core protein such that the GAG chain can align parallel or perpendicular to the axis of the collagen fibril (Weber *et al.* 1996). The side chain is able to interact with the side chain of another decorin molecule bound to a collagen molecule within an adjacent fibril, forming an interfibrillar bridge, shown schematically in Figure 2 (Scott 1996; Vesentini *et al.* 2005). Biglycan has one or two chondroitin or dermatan sulphate side chains and is thought to share a collagen binding site with decorin (Schönherr *et al.* 1995). Lumican and fibromodulin have 2 or 4 keratan side chains respectively (Yoon & Halper 2005) and share a binding site on type I collagen molecules, distinct from the decorin binding site (Yoon & Halper 2005; Kalamajski & Oldberg 2009).

Of the other known interfibrillar matrix components, COMP is the most abundant glycoprotein present in tendon (Smith *et al.* 1997). It is a large pentameric protein, consisting of 5 subunits arranged as arms around a central cylinder (DiCesare *et al.* 1994). Each subunit is able to bind to type I collagen at four sites on the collagen molecule (Rosenberg *et al.* 1998), and therefore, one COMP molecule can provide a link between 5 adjacent collagen molecules. Other glycoproteins present within the interfibrillar matrix include tenascin-C (Riley *et al.* 1996), which is comprised of six arms, bound together via their N-terminal interchain cross-linking domains (Jarvinen *et al.* 2000), and the transmembrane protein tenomodulin, which is also found in the interfibrillar matrix, where it may provide a link between the cells and surrounding matrix (Docheva *et al.* 2005).

Proteins of the interfascicular matrix

Previously, little attention has been given to the interfascicular matrix (IFM), but recent research suggests it has a specialized structure, with some proteins only present at this level. Proteins identified within the IFM include elastic fibres

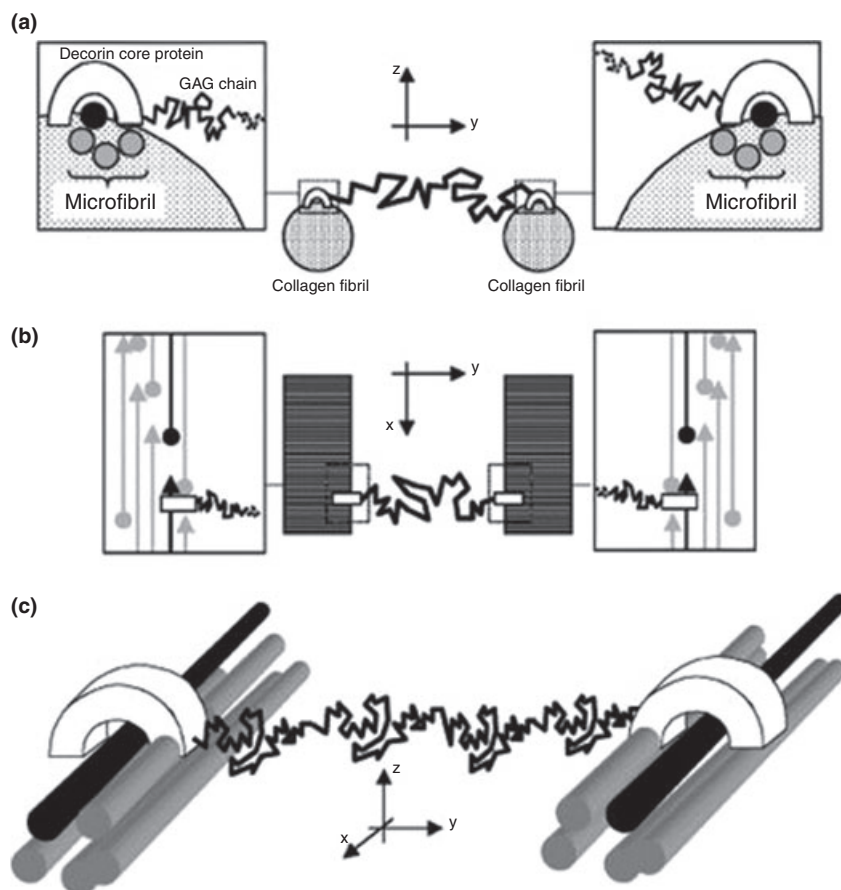


Figure 2 Schematic showing interaction between two collagen molecules from adjacent fibrils via decorin core proteins (white horseshoe shaped) and their glycosaminoglycan side chains (black lines). (a) Transverse view of collagen fibrils showing connections formed by decorin. (b) Longitudinal view of collagen fibrils showing decorin binding in the gap region of the collagen fibrils. (c) 3D representation of the decorin protein collagen molecule complex within a microfibril. Reprinted from Vesentini *et al.* (2005) with permission from Elsevier.

(Korol *et al.* 2007), lubricin (Sun *et al.* 2006), GAGs (Fallon *et al.* 2002), decorin (Kim *et al.* 2010), versican (Ritty *et al.* 2003) and collagen types III (Sodersten *et al.* 2013) and VI (Ritty *et al.* 2003), with an absence of COMP at this structural level (Sodersten *et al.* 2013).

The elastic fibre complex consists of a central core of elastin, surrounded by sheath of polymers of fibrillins 1 and 2, also known as microfibrils (Kielty *et al.* 2002). Many other molecules have been shown to associate with the elastic fibre, including decorin and biglycan (Baccarani-Contri *et al.* 1990). As its name suggests, elastin is a highly elastic protein that is able to extend by more than 100% of its original length (Aaron & Gosline 1981). It is formed from tropoelastin monomers that are coiled along their axis. This coil provides high elasticity with almost no hysteresis loss (Baldock *et al.* 2011). Further, elastin is highly fatigue resistant (Gosline *et al.* 2002; Lillie & Gosline 2002) and has the capacity for energy storage (Gosline *et al.* 2002). However, the precise role it plays within tendon is yet to be determined.

Elastin is reported to be present in tendon at concentrations in the range of 1–10% tendon dry weight (Silver *et al.* 2003; Korol *et al.* 2007). In the canine cruciate ligament, elastin has been shown to be situated between fascicles (Smith *et al.* 2011). Similarly, it has been shown that rat tail tendon fascicles are surrounded by a thin sheath of elastin (Korol *et al.* 2007). As well as colocalizing with elastin, fibrillins are also found independent of the elastic fibre; Ritty *et al.* (2003) identified fibrillin-2 tubules, with a uniform distribution in the IFM of the canine flexor digitorum profundus tendon. The role of these tubules is yet to be established, but it has been suggested that they may provide a framework to maintain cell linearity and enable cell migration (Ritty *et al.* 2003).

Another recently identified component of tendon matrix is lubricin. Lubricin is a large mucinous glycoprotein, also known as superficial zone protein or proteoglycan 4, and was originally identified within cartilage, where it acts as a boundary lubricant (Swann *et al.* 1981; Flannery *et al.* 1999). In tendon, lubricin was first shown to be present on the tendon surface, with greater levels in the compressive than tensional regions (Rees *et al.* 2002). It has been demonstrated that surface lubricin modulates tendon gliding resistance, allowing tendons to glide past one another or around joints (Taguchi *et al.* 2008, 2009). More recent work has shown that lubricin is also localized to the IFM within canine flexor tendon (Sun *et al.* 2008), caprine infraspinatus tendon (Funakoshi *et al.* 2008) and human supraspinatus tendon (Funakoshi *et al.* 2009).

The large proteoglycan versican has also been shown to be localized to the IFM and is found in greatest abundance in the pericellular region (Ritty *et al.* 2003). Versican is able to bind hyaluronic acid and the GAG chondroitin sulphate (Wight 2002) and has also been shown to interact with fibrillin-containing microfibrils in the elastic fibre (Isogai *et al.* 2002). These interactions suggest versican may contribute to the structural properties of the matrix by linking microfibrils to matrix components such as proteoglycans (Isogai *et al.* 2002), yet the precise role of versican within tendon remains undetermined.

Although the IFM mainly consists of non-collagenous components, collagen types III and VI are also present in low concentrations within this matrix. Whilst it is known that collagen type III content is increased in injured tendon (Birch *et al.* 1998; Sodersten *et al.* 2013), the function of this fibrillar collagen within the healthy IFM is yet to be established. Type VI collagen is non-fibrillar and is often found closely associated with cells (Senga *et al.* 1995; Ritty *et al.* 2003). Type VI collagen is able to bind to macromolecules including decorin and biglycan (Wiberg *et al.* 2001); these interactions may provide an anchoring network for cells within the IFM (Ritty *et al.* 2003).

The role of the non-collagenous matrix during development and maturation

Proteoglycans in developing tendon

The majority of studies on the NCM have focused on the role of NCM proteins during tendon development. This work has established that several non-collagenous proteins play an important role in modulating collagen fibrillogenesis. As decorin is the most abundant SLRP in the tensile region of tendon, this proteoglycan has been the most investigated. Decorin is involved in the correct alignment and stabilization of fibrils during fibrillogenesis (Scott 1996); it has been shown to inhibit lateral fusion of collagen fibrils; hence, high concentrations of decorin result in the formation of thinner collagen fibrils (Birk *et al.* 1995). Decorin-null mice exhibit severe tendon defects, characterized by the formation of highly disorganized collagen fibrils and localized areas of calcification within the tendon (Kilts *et al.* 2009). Decorin may also regulate the formation of enzymatic cross-links; the decorin binding site is in the C-terminal region of the collagen fibril and is in close proximity to the predominant site for intermolecular cross-link formation (Sweeney *et al.* 2008). These data suggest that decorin plays a crucial role in collagen fibrillogenesis in immature tendon.

Biglycan, like decorin, is able to bind to fibrillar collagen and has also been implicated in regulation of fibrillogenesis (Yoon & Halper 2005). However, the nature of biglycan-collagen interactions has not been fully determined (Zhang *et al.* 2005). Biglycan knockout mice exhibit similar defects to decorin knockouts, with thin, disorganized collagen fibrils and ectopic ossification, although they are not as severe in the biglycan knockout (Corsi *et al.* 2002; Kilts *et al.* 2009). Indeed, data indicate that decorin and biglycan have some similar functions as biglycan synthesis is upregulated in decorin-null mice, suggesting the mice may be able to partially compensate for the absence of decorin (Zhang *et al.* 2006).

Lumican and fibromodulin also interact with fibrillar collagens. These proteoglycans are differentially expressed during tendon development (Ezura *et al.* 2000), and it has been proposed that they are involved in the regulation of fibril fusion; *in vitro* studies have shown that they are able to inhibit fibril fusion (Hedbom & Heinegard 1989). Indeed, lumican- and fibromodulin-deficient mice exhibit tendon

defects characterized by larger, irregularly shaped fibrils, although these are not as severe as those seen in decorin or biglycan knockout models (Ezura *et al.* 2000).

Glycoproteins in developing tendon

Collagen oligomeric matrix protein interacts with collagen, cells and other matrix proteins (Smith *et al.* 2002b), and it is also thought to play a role in fibrillogenesis (Sodersten *et al.* 2005). In skeletally immature horses, the concentration of COMP is correlated with ultimate tensile stress and elastic modulus in the superficial digital flexor tendon (SDFT), suggesting that it plays an important role during tendon development (Smith *et al.* 2002b). In addition, mutations in the COMP gene are associated with pseudoachondroplasia, a disease characterized by joint laxity and bone abnormalities (Maddox *et al.* 2000). The mechanisms by which COMP mutations induce this disease are unknown, but it has been shown that COMP mutations affect proteoglycan synthesis in cartilage (Kwak *et al.* 2009). Knockout studies provide conflicting data, with one study unable to identify any tendon or musculoskeletal system abnormalities in COMP-null mice (Svensson *et al.* 2002). However, a more recent study has reported that Achilles tendons from COMP-null mice have a smaller cross section, larger fibril diameter and contain a greater number of bifurcated or fused fibrils than wild-type controls (Pirog *et al.* 2010), suggesting that this protein is also involved in collagen fibrillogenesis. Indeed, the structure of COMP, enabling it to bind 5 collagen molecules simultaneously, has been shown to promote collagen–collagen interactions and the formation of microfibrils (Halasz *et al.* 2007). Combined, these data suggest that COMP may work in conjunction with the SLRPs during maturation to ensure proper collagen fibrillogenesis.

Tenomodulin is also found in relatively high concentrations in immature tendon and has been shown to be essential for normal tenocyte proliferation. Tenomodulin is also involved in collagen fibril alignment and organization (Docheva *et al.* 2005), with tenomodulin-null mice exhibiting uneven fibril surfaces within their tendons (Docheva *et al.* 2005). Another extracellular matrix glycoprotein, tenascin-C, shows high expression in immature tendon and is also expressed in disease states and healing tissue (Riley *et al.* 1996; Jarvinen *et al.* 2000), but the roles of tenascin-C during tendon development and healing are yet to be defined.

Overall, these data indicate that a variety of tendon NCM proteins are required for proper collagen fibrillogenesis during development. Several of these proteins are able to regulate fibril diameter, alignment, stability and organization. This allows precise control of tendon growth and will ensure that appropriate mechanical properties are achieved.

The role of non-collagenous matrix in mature tendon

Whilst tendon NCM proteins clearly have an important role during growth and development, their function in mature

tendon is less well understood. With maturation, the levels of many NCM proteins tend to reduce (Jarvinen *et al.* 2000; Smith *et al.* 2002b; Shukunami *et al.* 2006; Zhang *et al.* 2006), and their roles become less well defined.

Proteoglycans in mature tendon

Several studies have investigated the role of SLRPs, particularly decorin, in mature tendon, and have suggested that this proteoglycan may contribute directly to tendon mechanical properties by aiding in the transfer of strain between discontinuous collagen fibrils via the interfibrillar bridges formed by the binding of one decorin side chain to another (Figure 2.). Individually, these bonds are weak (Vesentini *et al.* 2005), but combined, they may reach large enough magnitudes to transfer force between fibres. In support of this, computational simulations have shown that modelling collagen as discontinuous fibres, between which stress is transferred by decorin molecules and their GAG side chains, results in similar values for elastic modulus and ultimate stress as those recorded in mature tendon (Redaelli *et al.* 2003). However, there is controversy regarding the strength of the decorin protein and the interactions of this protein with collagen and other decorin molecule, whereby it has been postulated that the bonds formed would be too weak to contribute significantly to strain transfer (Provenzano & Vanderby 2006). Indeed, it has not been established if the discontinuous collagen fibril model is accurate or if fibrils span the length of the tendon, fusing to act as functionally continuous units (Provenzano & Vanderby 2006). It is clear that fibrils within immature tendon are discontinuous (Birk *et al.* 1995), and some tendons, including the equine SDFT and human Achilles, vary in cross-sectional area along their length (Birch *et al.* 2002), suggesting that not all fibrils run the entire length of the tendon. As such, these combined findings do suggest that fibrils within tendon are discontinuous, but do not confirm any role of decorin in force transfer between adjacent fascicles.

Indeed, the results of several enzymatic depletion studies have shown few alterations in tendon mechanical properties. In studies where chondroitinase has been used to remove GAG chains, in rat tail tendon fascicles (Fessel & Snedeker 2009, 2011) or human patellar tendon fascicles (Svensson *et al.* 2011), no changes in the failure properties were observed. With regard to gene knockout studies in mice, analysis of tendon mechanical properties in decorin-null mice did not identify differences in the maximum stress or modulus of tail tendon fascicles or flexor digitorum longus (FDL) tendons when compared with wild-type controls (Robinson *et al.* 2005).

Combined, these studies suggest that decorin (and other tendon proteoglycans) is unlikely to contribute directly to tendon ultimate properties. However, it has been reported that patellar tendons from decorin knockout mice have higher levels of stress relaxation when compared with controls (Robinson *et al.* 2005), whilst patellar tendons from decorin heterozygotes exhibit increased dynamic modulus

(Dourte *et al.* 2012). A very recent study has shown that GAG-depleted bovine extensor fascicles exhibit more stress relaxation and a greater reduction in failure stress after the application of static load (Legerlotz *et al.* 2013). In addition, tail tendon fascicles from decorin-deficient mice have been shown to have reduced sensitivity to the rate of applied strain (Robinson *et al.* 2004). These data suggest that proteoglycans may impart viscoelasticity without dramatically altering tendon material properties. It is well established that tendon extension occurs predominantly by sliding between adjacent fibres and fibrils, rather than by fibre/fibril extension (Arnoczky *et al.* 2002; Screen *et al.* 2004; Cheng & Screen 2007; Snedeker *et al.* 2009a,b; Goulam Houssen *et al.* 2011). It has been shown that levels of fibre sliding are greater in GAG-depleted rat tail fascicles compared with controls (Screen *et al.* 2005), and it has additionally been demonstrated that GAG-depleted murine Achilles tendons exhibit increased fibril sliding (Rigozzi *et al.* 2013). Treating rat tail fascicles with a decorin binding inhibitor has been shown to result in both increased fibre sliding (Wood *et al.* 2003) and tendon length, but does not result in alterations in tendon ultimate properties (Esther *et al.* 2008). These results suggest that decorin and potentially other SLRPs play an important role in modulating fibre sliding, a mechanism that results in the characteristic viscoelastic response observed in tendons. Alterations in viscoelastic properties are more likely to impact upon tendon fatigue properties rather than failure properties, therefore supporting previous studies that have shown no alterations in failure properties of GAG-depleted tendons (Fessel & Snedeker 2009, 2011; Svensson *et al.* 2011) but suggesting a change that may be of greater physiological importance.

Other non-collagenous proteins in mature tendon

Other glycoproteins may also influence sliding behaviour within mature tendon but their functions remain to be fully determined. Whilst COMP is the most abundant glycoprotein found within tendon (Smith *et al.* 1997), COMP levels

show no correlation with the failure properties of SDFTs from mature horses (Smith *et al.* 2002b). However, Pirog *et al.* (2010) have shown that Achilles tendons from COMP-null mice have a higher failure strain and exhibit greater stress relaxation during cyclic loading than tendons from wild-type controls. These data indicate that COMP may also influence fibre sliding within tendon.

At the fascicular level, the localization of lubricin to the interfascicular space has led to the hypothesis that this glycoprotein not only promotes tendon surface gliding, but also enables sliding between adjacent fascicles (Funakoshi *et al.* 2008). In support of this, mouse knockout studies have shown that tail tendon fascicle gliding resistance is greater in lubricin knockout mice than in wild-type mice (Kohrs *et al.* 2011). Elastin is also located between fascicles, and whilst no studies have directly determined the effect of elastin on tendon mechanical properties, it has been postulated that it contributes to tendon elasticity by being stretched in the toe region of the stress strain curve, before the collagen fibrils are loaded (Gosline *et al.* 2002). This is supported by histochemical studies that show the greatest distribution of elastic fibres occurs in the region of canine flexor digitorum profundus tendon that experiences the greatest strains (Ritty *et al.* 2002). Indeed, in canine cruciate ligament, it has been suggested that elastic fibres form the complex interconnections observed between fascicles, providing a passive recoil system to enable recovery after deformation (Smith *et al.* 2011). Further, similar interfascicular connections and distribution of elastin have recently been observed in the equine SDFT (Figure 3.). Lubricin and elastin may therefore work in combination to enable sliding and recoil between adjacent fascicles. However, little is known about the precise distribution and organization of these proteins, and their specific role in modulating tendon mechanical properties is yet to be determined.

In addition to ensuring correct tendon development, it is clear from these studies that NCM proteins also play an important role within mature tendon by modulating fibre and fascicle sliding and therefore influencing tendon

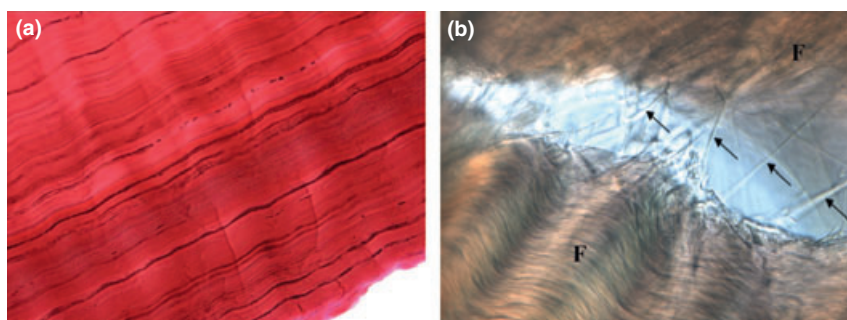


Figure 3 Images showing distribution of elastin and interfascicular connections in the equine superficial digital flexor tendon (SDFT). (a) Longitudinally sectioned SDFT stained with Miller's stain showing distribution of elastic fibres (black lines) between collagen bundles (E.G. Laird, unpublished data). (b) Nomarski differential interference contrast optical microscopy image of longitudinally cryosectioned SDFT showing interconnecting fibres (indicated by arrows) between adjacent fascicles (F) that have been teased apart (K.D. Smith, unpublished data).

viscoelastic properties. Absence or modulation of these molecules appears to result in excessive fibre sliding, which is likely to decrease the mechanical integrity of the tendon tissue, and result in increased risk of injury.

Differences in the non-collagenous matrix between tendon types

There is recent evidence showing that the structure and composition of the NCM differ between tendons with different functions, that is, those that are purely positional versus tendons with an additional function as energy stores. Examples of energy-storing tendons include the human Achilles tendon (Lichtwark & Wilson 2005) and the equine SDFT (Biewener 1998). These tendons must be able to extend and recoil efficiently and rapidly, with minimal energy loss, to maximize their energy-storing capacity (Lichtwark & Wilson 2007; Roberts & Azizi 2011). However, the high repetitive stresses and strains these tendons are exposed to make them highly prone to overstrain injury (Knobloch *et al.* 2008; Ely *et al.* 2009; Hess 2010). The equine SDFT is perhaps one of the most extreme examples of an energy-storing tendon; it experiences strains of up to 16% during gallop (Stephens *et al.* 1989) and may be exposed to strain rates as high as 200%/s (Herrick *et al.* 1978). There is little understanding of how these tendons are optimized for function; however, recent research suggests that the NCM is critical in facilitating these extremely high levels of extension and recoil in energy-storing tendons by enabling sliding between adjacent fascicles (Thorpe *et al.* 2012, 2013).

Several studies have compared and contrasted the composition and turnover of the NCM within the high-strain energy-storing equine SDFT and the low-strain positional common digital extensor tendon (CDET). It is well established that the SDFT has higher GAG levels than the CDET (Batson *et al.* 2003; Birch 2007), indicating a greater proteoglycan content. This is supported by our recent work that shows greater mRNA expression of a range of proteoglycans (aggrecan, biglycan, decorin, fibromodulin, lumican) in the SDFT than in the CDET (Thorpe 2010). It has also been shown previously that the half-life of the NCM is lower in the SDFT than in the CDET, demonstrating that turnover of this matrix occurs more rapidly in the SDFT (Thorpe *et al.* 2010b). In addition, gene expression data show greater expression of the stromelysins MMP-3 and MMP-10 in the SDFT when compared with the CDET (Thorpe 2010), as well as greater amounts of the pro- and active forms of MMP-3 at the protein level, as determined by casein zymography (Thorpe 2010). The stromelysin family of MMPs are able to degrade a variety of matrix molecules, including collagen type III, proteoglycans and elastin (Woessner 1991); these data therefore indicate greater metabolism of non-collagenous proteins in the energy-storing SDFT. There is further evidence showing a relatively high rate of proteoglycan turnover in flexor tendons from other species, with degradation products of biglycan and decorin identified within the bovine deep digital flexor tendon (Rees *et al.* 2000; Samiric

et al. 2004). This relatively high rate of NCM turnover in energy-storing tendons suggests that these proteins have a particularly important role to play in extension and recoil.

In addition to greater levels of proteoglycans in the equine SDFT, it has also been shown that COMP is present in greater abundance in this energy-storing tendon than in the positional CDET (Smith *et al.* 2002a). A similar relationship has been observed in human tendon, with higher COMP levels in the Achilles compared with the positional anterior tibialis tendon (Smith *et al.* 2002a).

These results show differences in NCM content and rate of turnover between tendons with different functions. The differential expression and turnover of non-collagenous proteins may affect tendon properties via several pathways. Greater levels of SLRPs will influence regulation of collagen fibrillogenesis in developing tendon and so are likely to be responsible for the lower mass average collagen fibril diameter observed in the SDFT compared with the CDET (Birch 2007). In addition, levels of sliding throughout the hierarchy will presumably be influenced by the composition of the NCM. This is supported by several studies showing that alterations in tendon non-collagenous proteins influence mechanical properties in a tendon specific manner. Enzymatic depletion and mice knockout studies have illustrated that the effect of decorin on tendon viscoelastic properties is specific to both tendon type and region (Robinson *et al.* 2004, 2005; Rigozzi *et al.* 2009). In addition, it has been illustrated that the effect of aggrecan content on mechanical properties may also be tendon specific; accumulation of aggrecan within the tensile region of the mouse FDL tendon in an ADAMTS-5 knockout model is associated with increased cross-sectional area and decreased modulus, whilst in the Achilles tendon, the opposite effect is observed, with an increase in modulus (Wang *et al.* 2012). This could be attributed to differences in structure between the tendons; the murine FDL is a single fascicle tendon, whereas the Achilles has a multifascicle structure (Wang *et al.* 2012). These data support the hypothesis that the function of non-collagenous proteins may not only be specific to tendon type and region, but also show specificity at a particular hierarchical level.

All these data indicate that the NCM plays a more important role in energy-storing tendons than in positional tendons. Indeed, we have very recent data that directly implicate the non-collagenous IFM in determining whole tendon mechanics. We have shown that fascicles from the SDFT have a lower failure strain than the whole tendon, with fascicles failing at strains below even the working range of the SDFT (Thorpe *et al.* 2012). However, we have established that there is a high capacity for sliding between adjacent fascicles and that the degree of sliding shows a positive correlation with whole tendon failure strain. By contrast, we observed little interfascicular sliding in the CDET, with small differences between tendon and fascicle failure strain, and no relationship between degree of sliding and tendon strain at failure (Thorpe *et al.* 2012). It has also been shown that there is the capacity for interfascicular

sliding in the human Achilles tendon (Haraldsson *et al.* 2008) and bovine deep digital flexor tendon (Purslow 2009), suggesting a similar mechanism for achieving high extension in these tendons. These data suggest that the non-collagenous IFM is specialized within energy-storing tendons to allow the greater extensions required by this tendon type whilst protecting the fascicles from damage. Whilst it has been shown that the area occupied by the IFM is greater in the equine SDFT than in the CDET (Thorpe *et al.* 2012), little is known about IFM structure and composition, and to the authors' knowledge, no studies have investigated IFM organization in functionally distinct tendons. Therefore, this represents an important area for future research.

Age-related alterations in the non-collagenous matrix

It is well established that the risk of injury to energy-storing tendons is increased in aged individuals (Kasashima *et al.* 2004; Perkins *et al.* 2005; Knobloch *et al.* 2008; Hess 2010). However, the majority of these studies have focused on the alterations to the collagenous matrix in aged tendons and relatively few studies have investigated age-related changes that occur to the tendon matrix. A few studies have documented changes that occur to the NCM with ageing, demonstrating age-related alterations in a variety of matrix proteins across several tendon types. The GAG content of rat patellar tendon has been shown to decrease with increasing age (Vailas *et al.* 1985), and GAG levels are also diminished in aged human supraspinatus tendon, but show no age-related decline in the common biceps tendon (Riley *et al.* 1994a). Collagen oligomeric matrix protein levels also show a tendon- and region-specific response to increasing age, with a decrease in COMP levels in the injury-prone tensional region of the equine SDFT, but no change in levels within the compressed region of this tendon or in the CDET (Smith *et al.* 1997, 2002b). In addition, it has been demonstrated that aged rat tail tendon has an increased elastin content, which is accompanied by decreased elastic modulus and failure stress (Vogel 1980). Whilst few studies have investigated changes in the levels of specific proteoglycans within aged tendon, it has been shown that a truncated form of decorin is present within aged skin (Carrino *et al.* 2000). If a similar accumulation occurs in aged tendon, it could result in altered collagen–proteoglycan interactions. This small body of research indicates that ageing results in alterations to tendon non-collagenous protein content and structure, which appears to be specific to both tendon type and region.

If the relationship between these compositional changes and mechanical properties is to be understood, we need to characterize how these changes influence tendon extension mechanisms. Indeed, our recent work demonstrates that the non-collagenous IFM shows age-related alterations in mechanical properties that are specific to tendon type. The stiffness of the IFM is increased in SDFTs from old horses; however, the IFM in the CDET does not show any age-related changes in mechanical properties (Thorpe *et al.*

2013). These findings suggest that the IFM is compromised in aged energy-storing SDFTs, resulting in reduced capacity for interfascicular sliding. A reduction in sliding between adjacent fascicles may result in a decrease in the ability of the tendon to respond to repetitive loading, potentially contributing to the increased risk of fatigue-induced injury with ageing. Whilst our data show that the area occupied by the IFM decreases with increasing age in the SDFT (Thorpe *et al.* 2013), the specific changes that occur to particular IFM components in aged tendons have not been investigated and so represent a further area for future study.

Conclusions

It is apparent that several significant advances have been made recently to further understand the role that non-collagenous proteins have in modulating tendon properties. However, a substantial amount of research is still required to develop a complete understanding of the role of each specific component at each hierarchical level and to determine the specializations that occur in tendons with differing mechanical demands. In addition, work needs to be undertaken to elucidate the changes that occur to this matrix with ageing and injury. This information is critical to fully understand structure–function relationships within tendon, which will aid in the development of novel, effective techniques for tendon injury prevention and treatment.

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Conflict of interest

The authors have no conflicts of interest.

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