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Tendon Functional Extracellular Matrix

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Abstract

This article is one of a series, summarising views expressed at the Orthopaedic Research Society New Frontiers in Tendon Research Conference. This particular article reviews the three workshops held under the "Functional Extracellular Matrix" stream. The workshops focused on the roles of the tendon extracellular matrix, such as performing the mechanical functions of tendon, creating the local cell environment and providing cellular cues. Tendon is a complex network of matrix and cells, and its biological functions are influenced by widely-varying extrinsic and intrinsic factors such as age, nutrition, exercise levels and biomechanics. Consequently, tendon adapts dynamically during development, ageing and injury. The workshop discussions identified research directions associated with understanding cell-matrix interactions to be of prime importance for developing novel strategies to target tendon healing or repair.

Keywords

tenocyte; cell; tendinopathy; collagen; proteoglycans; hierarchy; structure; mechanics

Overview

Tendons connect muscle to bone, carrying some of the highest forces experienced by any vertebrate tissue, as they facilitate movement and provide skeletal stability[1]. The ratio of matrix-to-cells in tendon is subsequently amongst the highest of any vertebrate tissues; thus

understanding matrix composition and organisation, and how cells interact with matrix, is key to understanding the function, homeostasis and repair of tendons. While significant advances in our understanding of tendon function have been made, major unanswered questions remain, such as how cells establish the tendon matrix and how the matrix organisation explains the mechanical properties of tendon. This knowledge is a prerequisite for the development of novel strategies to improve tendon repair in the treatment of tendinopathies.

Tendon composition

The extracellular matrix (ECM) of tendon is composed predominantly of collagen, which accounts for ~ 60–85% of the dry weight of the tissue[2]. Roughly 95% of the collagen is type I, with small levels of collagen types III, V, XI, XII and XIV[3; 4]. The collagen forms fibre-like structures at a number of different hierarchical levels, each aligned close to the long axis of the tissue (the loading direction), conferring excellent uniaxial mechanical strength to the tendon (figure 1). Collagen fibrils are the principle tensile element in tendon, and can be millimetres in length[5] and range in diameter from a few nanometers to over 300 nm[6]. Evidence from electron microscopy (e.g. see[7; 8]) suggests that collagen fibrils assemble at the plasma membrane of embryonic tenocytes[9; 10; 11], with the force required for transport coming from non-muscle myosin II[12]. However, further research is needed to fully establish the molecular and mechanical mechanisms. Collagen fibrils are grouped into fibres, fascicles and finally the whole tendon.

Interspersed between the collagen units throughout the tendon hierarchy is a variety of other non-collagenous matrix components[4]. Many of these are found across a range of other connective tissues; however details of the amounts, organisation and hierarchical locations of these non-collagenous matrix components are generally less well defined. Within tendon, it is unclear how these matrix components give tendon its unique properties, both mechanical and biological. However, in recent years, some progress has been made to understand their nature and function. The non-collagenous proteins can be grouped into proteoglycans, glycoproteins and glycoconjugates. Proteoglycans are generally divided into 1) large aggregating PGs such as versican and aggrecan and 2) members of the small leucine-rich proteoglycan (SLRP) family.

SLRPs are the abundant proteoglycans in tendon, with decorin accounting for roughly 80% of the total proteoglycan content of the tissue[13]. The SLRP family is composed of 17 members that are sub-divided into classes I-V based on their protein and DNA sequence homology[14]. Decorin (named because of its ability to decorate collagen fibrils[15]) is one of the most widely studied class I SLRPs in tendon, alongside biglycan[16; 17] (named because it contains two chains of attached glycosaminoglycans (GAGS)). The class II SLRPs fibromodulin[18] and lumican[13; 19; 20] are also present in tendon and, like decorin and biglycan, appear to have unique, but overlapping functions in fine-tuning collagen fibril assembly and subsequent tendon integrity[19; 20; 21; 22; 23; 24].

The large aggregating proteoglycans such as versican and aggrecan are particularly prominent in the pericellular regions[25], but also in compressive regions of tendon, for

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example where tendons wrap around joints[26]. Their role, increasing water content in these regions, provides resistance to compression[27]. The glycoproteins found in tendon include molecules such as lubricin[28], tenascin-C, collagen oligomeric matrix component[29] (COMP) and tenomodulin[30]. Elastic fibres, composed of elastin, fibrillins 1 and 2, as well as other elastic fibre-associated molecules are also present[31; 32]. In addition, microfibrillar structures containing fibrillins, not co-distributed with elastin, are localized throughout the tendon ECM[32]. Little is known about the function of these components. However, recent data has indicated that both lubricin and elastin are localised to the matrix between fascicles (the interfascicular matrix) where both may play a role in facilitating fascicle sliding and recoil[4; 28; 32; 33]. Lubricin is also found in the sheath around tendons, where it also may aid in sliding[34; 35]. The fibrillins have structural and instructive roles. The structural roles are dependent on the temporal and hierarchical assembly of elastic fibres and microfibrils. In contrast, the instructive roles are dependent on the ability of fibrillins to sequester transforming growth factor beta (TGFbeta) and bone morphogenetic protein (BMP) in the extracellular matrix[36].

The tendon cell population is heterogenous and poorly defined, with no clear markers available to identify the cells. At least two distinct cell populations are evident within tendon; the highly elongated specialised fibroblasts within fascicles (tenocytes)[37], that synthesise a collagen-rich extracellular matrix, and a population of more rounded cells in the interfascicular matrix, which appear more metabolically active[38]. A phenotypically different, active cell population also has been identified within the peritendon sheath[39]. Further analysis of these cells is necessary, to establish how they first establish, and subsequently maintain the tendon ECM. In addition, the lack of specific markers identifying cell type and functional state in tendons remains a major impediment to progress.

Tendon ECM Function

Like all connective tissues, tendon has a complex network of cells and surrounding ECM, performing a number of different functional roles. Appropriate composition and organisation of the ECM enables a tendon to perform its mechanical function of force transfer, but also ensures the biological function of tendon, maintaining the microenvironment to ensure cell and matrix health. Mechanical function and biological function are intrinsically linked and neither can be considered in isolation. However, different disciplines tend to use the word "function" generically, and multidisciplinary discussions may benefit from a clear articulation of "biological function" or "mechanical function" when reviewing the role of the matrix.

Mechanical Function

Tendons are predominantly loaded along their long axis in tension, enabling muscles to move the skeleton to position the body. While all tendons perform this positional role, some tendons have an additional role, stretching when loaded to store energy, which they can later return to the system to improve the efficiency of locomotion[40]. Such tendons are called energy storing tendons. Positional tendons are generally subjected to small strains, in the region of 2–3% in vivo, whilst energy storing tendons can experience strains in excess of

10% during use[41; 42]. Unsurprisingly, this more demanding mechanical environment leaves energy storing tendons more prone to injuries, termed tendinopathies.

An in-vitro analysis of tendon mechanical properties has identified differences in both the quasi-static and time-dependent mechanical properties of energy storing and positional tendons as befits their functional roles. Energy storing tendons are less stiff and more extensible than positional tendons[42; 43], and also behave more elastically, showing less hysteresis and time dependent behaviour[44; 45]. An overview of differences in energy storing and positional tendon mechanical parameters is provided in figure 2.

It is notable that much of the data comparing energy storing and positional tendons has focused on a comparison between the superficial digital flexor tendon (SDFT) and common digital extensor tendon (CDET) of the equine hoof. The SDFT provides a good model for the human Achilles tendon, prone to aetiologically similar tendinopathies[47], but is also a particularly highly loaded energy storing tendon[40], providing an excellent structure-function model to study the structural mechanisms enabling the tendon to perform its mechanical function.

Compositional differences between energy storing and positional tendons have been reported. For example, the energy storing SDFT has higher levels of GAG than the positional CDET indicating a greater proteoglycan content[41; 43]. It also has a greater abundance of COMP[29], and collagen crosslinking also differs, with the mature crosslink hydroxylysinonorleucine (HHL) found only in the positional CDET[48]. More recent studies have attempted to localise the compositional differences between tendon types, with the aim of establishing how specific compositional or structural variations contribute to different tendon mechanical or biological behaviours. From a mechanics perspective, a tendon can be considered as a composite material, comprised of fibrous collagen units surrounded by non-collagenous matrix, at multiple hierarchical levels. The transfer of strain through this complex multilevel structure is subsequently highly inhomogenous, involving not just direct loading and extension of the collagen units, but also shearing or sliding between different levels of the collagen hierarchy, modulated by the non-collagenous matrix. Small local variations in the composition of the non-collagenous matrix at a single hierarchical level within the tendon can subsequently have a significant impact on tendon mechanical behaviour. These variations are, as yet, poorly understood, but data indicate that in positional tendons, proteoglycans located between fibrils enable fibril sliding[49; 50; 51] and result in a more viscoelastic tendon behaviour, whilst energy storing tendons rely on lubricin and elastin between fascicles to enable a more elastic, recoverable fascicle sliding[4; 42; 52]. Energy storing tendon fascicles also appear to be helically arranged, contributing to a spring-like, elastic behaviour[44; 45]. Further work is necessary to understand how tendon structure is optimised to provide appropriate mechanical function across tendon types, and identify the specific matrix components / organisations providing that mechanical behaviour.

Biological Function

It is well known that cell-matrix interactions are dynamic, and connective tissues will not only turnover in homeostatic conditions to maintain health, but also will remodel in response

to different stimuli. There is some evidence that these processes can be adaptive, and whilst the speed of adaptation is slow, tendon can thicken and strengthen in response to use[53; 54]. However, both overuse and underuse have been reported to initiate a more rapid catabolic cell response and tendon degeneration[38; 55; 56; 57].

Investigating cell-matrix interactions is complex, as the relationships are not just dynamic, but are also cyclically linked, directly influencing one another. Tendon cells govern the production and organisation of the tendon matrix in response to mechanical and chemical cues[58]. However, the structure and composition of the matrix is directly responsible for controlling the cues reaching the cells[59], generating complex networks and feedback loops linking cell and matrix fate within the tendon. To provide some examples, the mechanical load placed on a tendon when walking will lead to inhomogeneous strains throughout the tendon matrix, and it is these local strains that are perceived at a cellular level[60]. Matrix turnover is modulated in response to these local strains, adapting the matrix, so the same external load stimulus may be perceived very differently at the cell level over time. A similar dynamic relationship may occur with biological stimuli. The tendon ECM may act to manipulate local availability of the growth factor at a cell level, adjusting cell metabolism and subsequently influencing local matrix conditions and future availability of the growth factor[61]. The influence of individual matrix components in modulating growth factors may have multiple outcomes, including the regulation of stem cell maintenance, propagation and overall fate[62]. A major limitation in deepening our understanding in this area is the paucity of ways to track and monitor tendon progenitors[63]. In this regard, a systematic exploration of tendon cell character is needed. With new tendon markers in hand, the fate and function of tendon cells during development, aging and in disease and, in particular, the role of the ECM in these processes could be seriously addressed.

Dynamic and cyclic cell-matrix interactions are poorly understood in isolation, yet we have even less understanding of how they interact. Research building our understanding of dynamic cell-matrix interactions is critical to provide a cornerstone towards understanding functional extracellular matrix. To further complicate the issue, not only are cell-matrix interactions dynamic processes, but they must be considered in a temporal manner. The circadian cycle affects matrix homeostasis, whilst periods of mechanical stimulus associated with loading a tendon and rest create variable temporal stimulus patterns. The circadian clock regulates gene expression in anticipation of an expected environmental change[64]. Therefore it is no surprise that bone[65], muscle[66], cartilage[67] and tendon[68] are prominent peripheral clock tissues in which the expression of specific sets of genes are regulated in readiness for diurnal mechanical activity. In tendon, BMP signalling and the suppression of calcification is under strict circadian control[68]. Hormonal changes can influence tissues[69], whilst nutritional and lifestyle factors can influence cell-matrix interactions and matrix metabolism over time-spans in the range of months or even years[70].

Furthermore, recent data indicates that turnover rates differ between matrix components[48], and whilst collagenous components have an exceptionally long half-life (in the hundreds of years[71]) and show little change during a life time, the non-collagenous components of the matrix are able to turn over and adapt to new stimuli much faster[48], perhaps another indicator of their importance within tendon.

Tendinopathy

Tendinopathy is the term given to a broad spectrum of clinical tendon disorders, but is most commonly concerned with chronic painful tendon conditions, in which matrix degradation, neovascularisation and swelling are evident[72; 73]. The initiation and development of tendinopathy is a temporal and multifactorial process, likely initiated by overuse. However, it remains unclear if the initial driving factor towards tendinopathy is matrix disruption generated by mechanical overuse or a cellular response to altered loading conditions. It is likely that both factors are implicated, with local mechanical matrix damage initiating a biological cascade of events culminating in matrix deterioration[74; 75]. However, multiple extrinsic and intrinsic factors will influence the dynamic processes of matrix metabolism in health as well as propensity to injury, and will influence cell-matrix interactions in response to overuse.

Interrogating the aeitiology of tendinopathy presents a series of methodological difficulties. Any analysis of human tendinopathic tissue occurs in late stage disease, when matrix changes are chronic. These data have been highly beneficial in understanding chronic, degenerative tendon conditions[76; 77], but with no controlled initiation or development of the tendinopathy and only late stage data for analysis, it is difficult to interrogate early tendinopathy. Animal models provide a mechanism for investigating the acute stages of tendinopathy, and more fully characterising temporal aspects of its aetiology[78]. However, the high degree of heterogeneity in structure, function and metabolism of tendons, both across species and functionally distinct tendons can make the resulting data difficult to interpret.

Questions associated with tendon matrix healthy function and the aetiology of tendinopathy are general complex and multifactorial. In order to begin unravelling these, it is necessary to select a model system in which to undertake highly controlled studies, to investigate a subset of the biological and mechanical parameters involved in these behaviours. Such studies are undoubtedly contributing to our understanding of tendon and the aetiology of tendinopathy, advancing our knowledge base. For example, recent data has begun to quantify the development of matrix disruption with tendon overuse, correlating this to reduced tendon mechanics[79]. Early overuse damage has been identified within the non-collagenous regions of tendon, and overuse shown to initiate an immediate inflammatory response in tendon[38], alongside an upregulation of matrix degradation and cell apoptosis[80]. However, whilst these data provide important insights into tendinopathy, we still lack a functionally ideal model to enhance further studies. While a single perfect model is probably not attainable, further model development and characterization is a high priority. During this process, we must not forget the full spectrum of complex interactions between cell and matrix and the need for multidisciplinary approaches to interrogate tendon mechanobiology.

Future Directions

There remain multiple unanswered questions associated with ECM function in health and disease, and a number of key avenues for further exploration. We need to establish the molecular and mechanical mechanisms associated with how the ECM develops, and carry

out a systemic exploration of the different tendon cell phenotypes, to establish their roles in development, health and disease. To this end, future studies characterizing markers for cell type and functional state will be critical. In addition, the development of animal and cell based models will be necessary to elevate these studies to the next level. We also need a more detailed understanding of both the interactions between matrix components and the interactions between cells and matrix, across the multiple hierarchical levels of the tendon ECM, to establish their biological and mechanical implications. This will facilitate efforts to establish how ECM structure provides mechanical function and also regulates dynamic cell-matrix interactions.

Characterising these behaviours in health provides a basis from which to investigate the aetiology of tendinopathy, and the processes governing ECM degeneration in disease. As part of these efforts, further development and characterisation of tendinopathy models is high priority[81].

To continue progress and increased understanding of tendon biology and pathobiology, it is important to embrace multidisciplinary approaches and work in collaborative teams to identify the contributions of cells, matrix, and mechanical forces to achieve tendon biological and mechanical function. The ability to communicate more effectively across disciplines is critical and requires researchers and clinicians to move out of their traditional research focuses and embrace new approaches. We have an opportunity to support this learning as we train early career researchers; exposure to multiple laboratories, techniques and scientific viewpoints during training will help support effective cross-discipline work.

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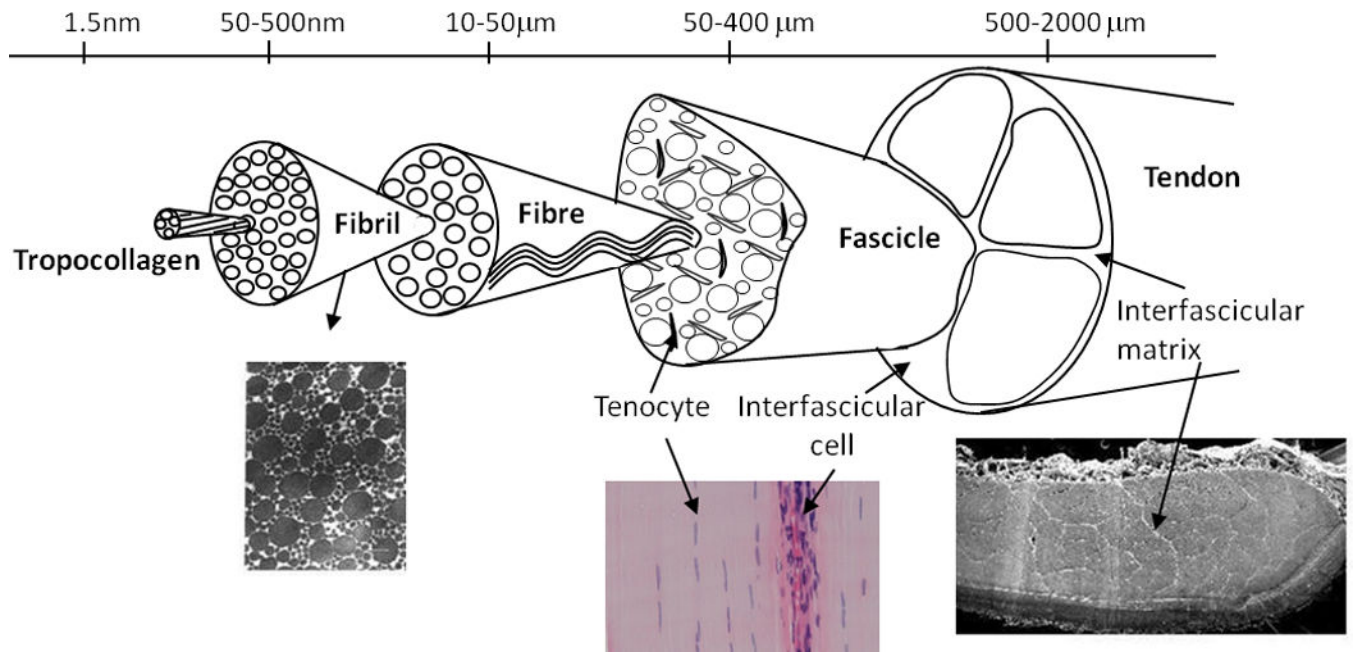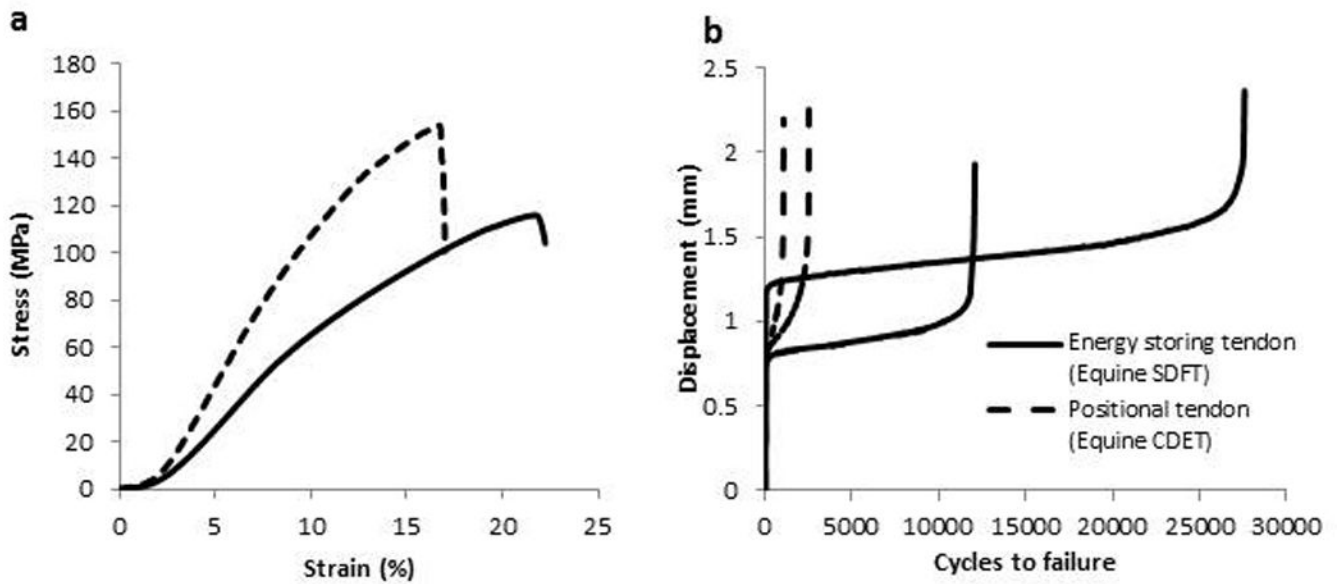
Figure 1.
Schematic depicting the hierarchical structure of tendon, with inset images: Transverse sections show fibril and fascicle packing. The longitudinal histological section (H&E) shows the tendon cell populations.

Figure 2.

Quasi-static and time dependent mechanical properties differ between energy storing and positional tendons. Typical data in parts a and b highlight differences in (a) the quasi-static properties and (b) the time dependent properties of the equine superficial digital flexor tendon (SDFT - energy storing tendon - solid line) and the common digital extensor tendon (CDET - positional tendon - dotted line). The table (c) provides a more comprehensive overview of data from a series of studies comparing paired equine SDFT & CDET tendons. Note the less stiff, more extensible and more fatigue resistant energy storing tendon properties[42; 44; 45; 46].