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Tendon Functional Extracellular Matrix**Screen H.R.C.¹, Birk D.E.², Kadler K.E.³, Ramirez F⁴, Young M.F.⁵**

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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, held at the Icahn School of Medicine at Mount Sinai, New York, September 2014. 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 stability¹. 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 tissue². 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 length⁵ and range in diameter from a few nanometers to over 300 nm⁶.

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 II¹². 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.

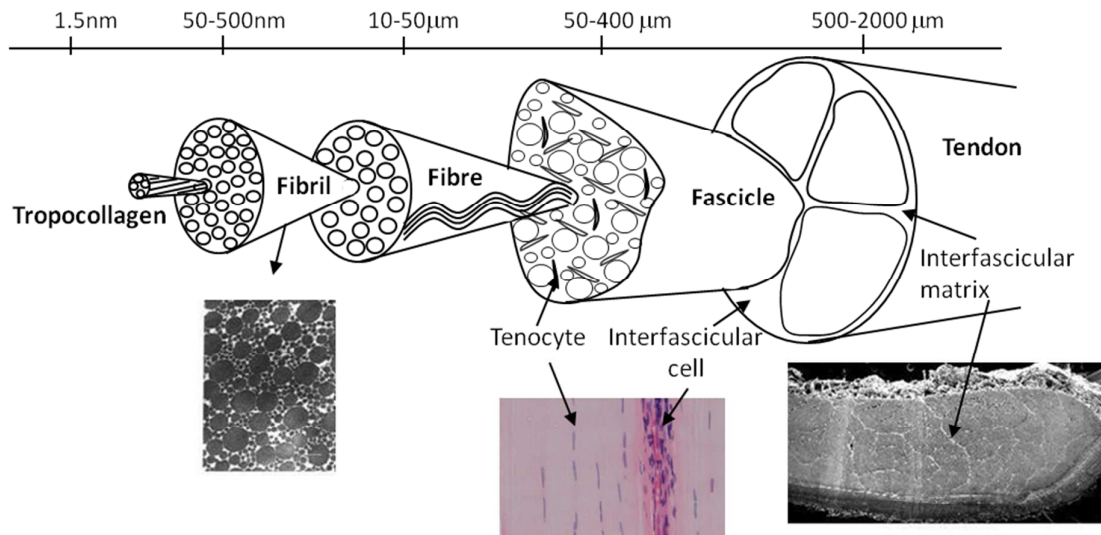


Figure 1: Schematic depicting the hierarchical structure of tendon, with inset images: Transverse sections show fibril and fascicle packing. The longitudinal histological section (H&E) shows the tendon cell populations.

Interspersed between the collagen units throughout the tendon hierarchy is a variety of other non-collagenous matrix components⁴. 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 tissue¹³. The SLRP family is composed of 17 members

1
2
3 that are sub-divided into classes I-V based on their protein and DNA sequence homology¹⁴.
4
5 Decorin (named because of its ability to decorate collagen fibrils¹⁵) is one of the most widely
6
7 studied class I SLRPs in tendon, alongside biglycan^{16; 17} (named because it contains two
8
9 chains of attached glycosaminoglycans (GAGS)). The class II SLRPs fibromodulin¹⁸ and
10
11 lumican^{13; 19; 20} are also present in tendon and, like decorin and biglycan, appear to have
12
13 unique, but overlapping functions in fine-tuning collagen fibril assembly and subsequent
14
15 tendon integrity^{19; 20; 21; 22; 23; 24}.

16
17
18 The large aggregating proteoglycans such as versican and aggrecan are particularly
19
20 prominent in the pericellular regions²⁵, but also in compressive regions of tendon, for
21
22 example where tendons wrap around joints²⁶. Their role, increasing water content in these
23
24 regions, provides resistance to compression²⁷. The glycoproteins found in tendon include
25
26 molecules such as lubricin²⁸, tenascin-C, collagen oligomeric matrix component²⁹ (COMP)
27
28 and tenomodulin³⁰. Elastic fibres, composed of elastin, fibrillins 1 and 2, as well as other
29
30 elastic fibre-associated molecules are also present^{31; 32}. In addition, microfibrillar structures
31
32 containing fibrillins, not co-distributed with elastin, are localized throughout the tendon
33
34 ECM³². Little is known about the function of these components. However, recent data has
35
36 indicated that both lubricin and elastin are localised to the matrix between fascicles (the
37
38 interfascicular matrix) where both may play a role in facilitating fascicle sliding and recoil^{4;}
39
40 ^{28; 32; 33}. Lubricin is also found in the sheath around tendons, where it also may aid in sliding^{34;}
41
42 ³⁵. The fibrillins have structural and instructive roles. The structural roles are dependent on
43
44 the temporal and hierarchical assembly of elastic fibres and microfibrils. In contrast, the
45
46 instructive roles are dependent on the ability of fibrillins to sequester transforming growth
47
48 factor beta (TGFbeta) and bone morphogenetic protein (BMP) in the extracellular matrix³⁶.
49
50 The tendon cell population is heterogenous and poorly defined, with no clear markers
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52 available to identify the cells. At least two distinct cell populations are evident within tendon;
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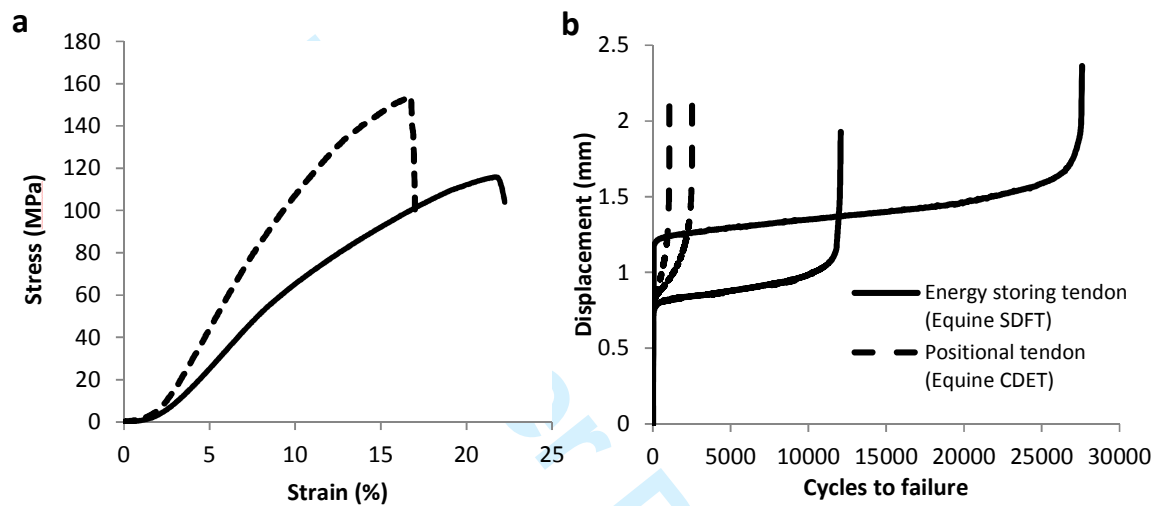
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2
3 the highly elongated specialised fibroblasts within fascicles (tenocytes)³⁷, that synthesise a
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5 collagen-rich extracellular matrix, and a population of more rounded cells in the
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7 interfascicular matrix, which appear more metabolically active³⁸. A phenotypically different,
8
9 active cell population also has been identified within the peritendon sheath³⁹. Further analysis
10
11 of these cells is necessary, to establish how they first establish, and subsequently maintain the
12
13 tendon ECM. In addition, the lack of specific markers identifying cell type and functional
14
15 state in tendons remains a major impediment to progress.
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18
19

20 21 **Tendon ECM Function:**

22
23 Like all connective tissues, tendon has a complex network of cells and surrounding ECM,
24
25 performing a number of different functional roles. Appropriate composition and organisation
26
27 of the ECM enables a tendon to perform its mechanical function of force transfer, but also
28
29 ensures the biological function of tendon, maintaining the microenvironment to ensure cell
30
31 and matrix health. Mechanical function and biological function are intrinsically linked and
32
33 neither can be considered in isolation. However, different disciplines tend to use the word
34
35 "function" generically, and multidisciplinary discussions may benefit from a clear articulation
36
37 of "biological function" or "mechanical function" when reviewing the role of the matrix.
38
39

40
41 **Mechanical Function:** Tendons are predominantly loaded along their long axis in tension,
42
43 enabling muscles to move the skeleton to position the body. While all tendons perform this
44
45 positional role, some tendons have an additional role, stretching when loaded to store energy,
46
47 which they can later return to the system to improve the efficiency of locomotion⁴⁰. Such
48
49 tendons are called energy storing tendons. Positional tendons are generally subjected to small
50
51 strains, in the region of 2-3% in vivo, whilst energy storing tendons can experience strains in
52
53 excess of 10% during use^{41; 42}. Unsurprisingly, this more demanding mechanical environment
54
55 leaves energy storing tendons more prone to injuries, termed tendinopathies.
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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**.



c

Mechanical Properties		Positional Tendons	Energy Storing Tendons
Quasi-static Properties	Failure Stress	120-200 MPa	90-145 MPa
	Failure Strain	15-22 %	18-28 %
	Modulus	800-1200 MPa	500-700 MPa
Cyclic Stress relaxation (10 cycles to 25% of failure strain)	Stress Relaxation	11-18 %	10-14 %
	Hysteresis loss	35-55 %	20-40 %
Cyclic Creep (to stress at 60% of failure strain)	Creep cycles to failure	200-3000	3000-25000

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

1
2
3 **common digital extensor tendon (CDET - positional tendon - dotted line). The table (c)**
4
5 **provides a more comprehensive overview of data from a series of studies comparing**
6
7 **paired equine SDFT & CDET tendons. Note the less stiff, more extensible and more**
8
9 **fatigue resistant energy storing tendon properties**^{42; 44; 45; 46}.
10
11

12
13
14 It is notable that much of the data comparing energy storing and positional tendons has
15
16 focused on a comparison between the superficial digital flexor tendon (SDFT) and common
17
18 digital extensor tendon (CDET) of the equine hoof. The SDFT provides a good model for the
19
20 human Achilles tendon, prone to aetiologically similar tendinopathies⁴⁷, but is also a
21
22 particularly highly loaded energy storing tendon⁴⁰, providing an excellent structure-function
23
24 model to study the structural mechanisms enabling the tendon to perform its mechanical
25
26 function.
27

28
29 Compositional differences between energy storing and positional tendons have been reported.
30
31 For example, the energy storing SDFT has higher levels of GAG than the positional CDET
32
33 indicating a greater proteoglycan content^{41; 43}. It also has a greater abundance of COMP²⁹,
34
35 and collagen crosslinking also differs, with the mature crosslink hydroxylysinoxorleucine
36
37 (HHL) found only in the positional CDET⁴⁸. More recent studies have attempted to localise
38
39 the compositional differences between tendon types, with the aim of establishing how
40
41 specific compositional or structural variations contribute to different tendon mechanical or
42
43 biological behaviours. From a mechanics perspective, a tendon can be considered as a
44
45 composite material, comprised of fibrous collagen units surrounded by non-collagenous
46
47 matrix, at multiple hierarchical levels. The transfer of strain through this complex multilevel
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49 structure is subsequently highly inhomogenous, involving not just direct loading and
50
51 extension of the collagen units, but also shearing or sliding between different levels of the
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53 collagen hierarchy, modulated by the non-collagenous matrix. Small local variations in the
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3 composition of the non-collagenous matrix at a single hierarchical level within the tendon can
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5 subsequently have a significant impact on tendon mechanical behaviour. These variations are,
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7 as yet, poorly understood, but data indicate that in positional tendons, proteoglycans located
8
9 between fibrils enable fibril sliding^{49; 50; 51} and result in a more viscoelastic tendon behaviour,
10
11 whilst energy storing tendons rely on lubricin and elastin between fascicles to enable a more
12
13 elastic, recoverable fascicle sliding^{4; 42; 52}. Energy storing tendon fascicles also appear to be
14
15 helically arranged, contributing to a spring-like, elastic behaviour^{44; 45}. Further work is
16
17 necessary to understand how tendon structure is optimised to provide appropriate mechanical
18
19 function across tendon types, and identify the specific matrix components / organisations
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21 providing that mechanical behaviour.
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25
26

27 **Biological Function:**

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29 It is well known that cell-matrix interactions are dynamic, and connective tissues will not
30
31 only turnover in homeostatic conditions to maintain health, but also will remodel in response
32
33 to different stimuli. There is some evidence that these processes can be adaptive, and whilst
34
35 the speed of adaptation is slow, tendon can thicken and strengthen in response to use^{53; 54}.
36
37 However, both overuse and underuse have been reported to initiate a more rapid catabolic
38
39 cell response and tendon degeneration^{38; 55; 56; 57}.
40
41

42
43 Investigating cell-matrix interactions is complex, as the relationships are not just dynamic,
44
45 but are also cyclically linked, directly influencing one another. Tendon cells govern the
46
47 production and organisation of the tendon matrix in response to mechanical and chemical
48
49 cues⁵⁸. However, the structure and composition of the matrix is directly responsible for
50
51 controlling the cues reaching the cells⁵⁹, generating complex networks and feedback loops
52
53 linking cell and matrix fate within the tendon. To provide some examples, the mechanical
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55 load placed on a tendon when walking will lead to inhomogeneous strains throughout the
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1
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3 tendon matrix, and it is these local strains that are perceived at a cellular level⁶⁰. Matrix
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5 turnover is modulated in response to these local strains, adapting the matrix, so the same
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7 external load stimulus may be perceived very differently at the cell level over time. A similar
8
9 dynamic relationship may occur with biological stimuli. The tendon ECM may act to
10
11 manipulate local availability of the growth factor at a cell level, adjusting cell metabolism
12
13 and subsequently influencing local matrix conditions and future availability of the growth
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15 factor⁶¹. The influence of individual matrix components in modulating growth factors may
16
17 have multiple outcomes, including the regulation of stem cell maintenance, propagation and
18
19 overall fate⁶². A major limitation in deepening our understanding in this area is the paucity of
20
21 ways to track and monitor tendon progenitors⁶³. In this regard, a systematic exploration of
22
23 tendon cell character is needed. With new tendon markers in hand, the fate and function of
24
25 tendon cells during development, aging and in disease and, in particular, the role of the ECM
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27 in these processes could be seriously addressed.
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32 Dynamic and cyclic cell-matrix interactions are poorly understood in isolation, yet we have
33
34 even less understanding of how they interact. Research building our understanding of
35
36 dynamic cell-matrix interactions is critical to provide a cornerstone towards understanding
37
38 functional extracellular matrix. To further complicate the issue, not only are cell-matrix
39
40 interactions dynamic processes, but they must be considered in a temporal manner. The
41
42 circadian cycle affects matrix homeostasis, whilst periods of mechanical stimulus associated
43
44 with loading a tendon and rest create variable temporal stimulus patterns. The circadian clock
45
46 regulates gene expression in anticipation of an expected environmental change⁶⁴. Therefore
47
48 it is no surprise that bone⁶⁵, muscle⁶⁶, cartilage⁶⁷ and tendon⁶⁸ are prominent peripheral clock
49
50 tissues in which the expression of specific sets of genes are regulated in readiness for diurnal
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52 mechanical activity. In tendon, BMP signalling and the suppression of calcification is under
53
54 strict circadian control⁶⁸. Hormonal changes can influence tissues⁶⁹, whilst nutritional and
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1
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3 lifestyle factors can influence cell-matrix interactions and matrix metabolism over time-spans
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5 in the range of months or even years⁷⁰.

6
7 Furthermore, recent data indicates that turnover rates differ between matrix components⁴⁸,
8
9 and whilst collagenous components have an exceptionally long half-life (in the hundreds of
10
11 years⁷¹) and show little change during a life time, the non-collagenous components of the
12
13 matrix are able to turn over and adapt to new stimuli much faster⁴⁸, perhaps another indicator
14
15 of their importance within tendon.
16
17

20 21 **Tendinopathy:**

22
23 Tendinopathy is the term given to a broad spectrum of clinical tendon disorders, but is most
24
25 commonly concerned with chronic painful tendon conditions, in which matrix degradation,
26
27 neovascularisation and swelling are evident^{72; 73}. The initiation and development of
28
29 tendinopathy is a temporal and multifactorial process, likely initiated by overuse. However, it
30
31 remains unclear if the initial driving factor towards tendinopathy is matrix disruption
32
33 generated by mechanical overuse or a cellular response to altered loading conditions. It is
34
35 likely that both factors are implicated, with local mechanical matrix damage initiating a
36
37 biological cascade of events culminating in matrix deterioration^{74; 75}. However, multiple
38
39 extrinsic and intrinsic factors will influence the dynamic processes of matrix metabolism in
40
41 health as well as propensity to injury, and will influence cell-matrix interactions in response
42
43 to overuse.
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47 Interrogating the aetiology of tendinopathy presents a series of methodological difficulties.
48
49 Any analysis of human tendinopathic tissue occurs in late stage disease, when matrix changes
50
51 are chronic. These data have been highly beneficial in understanding chronic, degenerative
52
53 tendon conditions^{76; 77}, but with no controlled initiation or development of the tendinopathy
54
55 and only late stage data for analysis, it is difficult to interrogate early tendinopathy. Animal
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2
3 models provide a mechanism for investigating the acute stages of tendinopathy, and more
4
5 fully characterising temporal aspects of its aetiology⁷⁸. However, the high degree of
6
7 heterogeneity in structure, function and metabolism of tendons, both across species and
8
9 functionally distinct tendons can make the resulting data difficult to interpret.
10
11 Questions associated with tendon matrix healthy function and the aetiology of tendinopathy
12
13 are general complex and multifactorial. In order to begin unravelling these, it is necessary to
14
15 select a model system in which to undertake highly controlled studies, to investigate a subset
16
17 of the biological and mechanical parameters involved in these behaviours. Such studies are
18
19 undoubtedly contributing to our understanding of tendon and the aetiology of tendinopathy,
20
21 advancing our knowledge base. For example, recent data has begun to quantify the
22
23 development of matrix disruption with tendon overuse, correlating this to reduced tendon
24
25 mechanics⁷⁹. Early overuse damage has been identified within the non-collagenous regions of
26
27 tendon, and overuse shown to initiate an immediate inflammatory response in tendon³⁸,
28
29 alongside an upregulation of matrix degradation and cell apoptosis⁸⁰. However, whilst these
30
31 data provide important insights into tendinopathy, we still lack a functionally ideal model to
32
33 enhance further studies. While a single perfect model is probably not attainable, further
34
35 model development and characterization is a high priority. During this process, we must not
36
37 forget the full spectrum of complex interactions between cell and matrix and the need for
38
39 multidisciplinary approaches to interrogate tendon mechanobiology.
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48 **Future Directions:**

49 To progress our understanding of ECM function in health and disease, it is important to
50
51 embrace multidisciplinary approaches and work in collaborative teams to identify the
52
53 contributions of cells, matrix, and mechanical forces to achieve tendon biological and
54
55 mechanical function. The ability to communicate more effectively across disciplines is
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1
2
3 critical and requires researchers and clinicians to move out of their traditional research
4
5 focuses and embrace new approaches. We have an opportunity to support this learning as we
6
7 train early career researchers; exposure to multiple laboratories, techniques and scientific
8
9 viewpoints during training will help support effective cross-discipline work.
10
11

12
13
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19

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