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REVIEW ARTICLE

Tendon Mechanobiology: Experimental Models Require Mathematical Underpinning

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Abstract Mathematical and computational modeling is in demand to help address current challenges in mechanobiology of musculoskeletal tissues. In particular for tendon, the high clinical importance of the tissue, the huge mechanical demands placed on it and its ability to adapt to these demands, require coupled, multiscale models incorporating complex geometrical and microstructural information as well as time-based descriptions of cellular activity and response.

This review introduces the information sources required to develop such multiscale models. It covers tissue structure and biomechanics, cell biomechanics, the current understanding of tendon's ability in health and disease to update its properties and structure and the few already existing multiscale mechanobiological models of the tissue. Finally, a sketch is provided of what such models could achieve ideally, pointing out where experimental data and knowledge are still missing.

Keywords Mechanobiology · Tendon · Multiscale modeling · Microstructure · Mechanical stimuli

1 Scientific and Clinical Motivation

As the global population ages and the benefits of regular physical exercise are becoming increasingly clear, musculoskeletal health is highlighted as essential for a high quality of life and healthy aging. Musculoskeletal tissues not only fulfil mechanical functions, but also rely on mechanical loading to maintain their healthy function through a homeostatic mechanobiological feedback loop (Fig. 1). Understanding the link between the mechanical environment, as provided by normal everyday life, and

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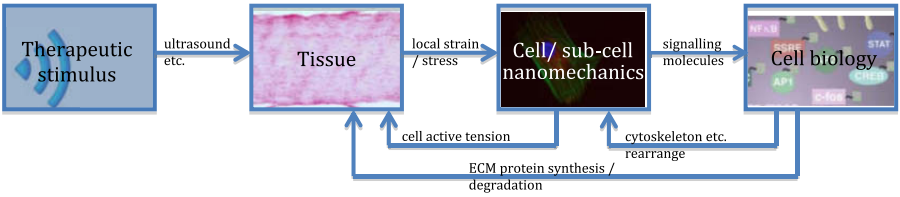

Fig. 1 Therapeutic mechanotransduction as a transfer function block diagram. The therapeutic stimulus is modified by transmission through tissue and across length scales, and by bio-feedback mechanisms

the cell-level events of tissue maintenance, repair, and new synthesis, is key to diagnosing, treating, and preventing painful and costly disorders of the musculoskeletal system.

Amongst musculoskeletal tissues, tendon has a vital role to play in enabling locomotion. Tendon disorders and disease have a major impact on individual pain and suffering and on society due to both the costs of frequently unsuccessful treatment and lost days at work.

Mathematical and computational models can capture and crystallize the current state of the art of mechanical and biological understanding of physiological and pathological processes. They give easy access to parameters that are impossible or difficult to measure in patients non-invasively. These models can couple multiple length and time scales together, and in systems of sufficient complexity are able to predict emergent behavior. Models develop iteratively as they test hypotheses from or are tested by other experimental systems, and as new information becomes available about the "real" system, the patient.

The focus of this review is on the existing experimental mechanobiological modeling in tendon, and highlights the need for more comprehensive mathematical treatment of this system. It begins with an overview of the basic physiology, microstructure, and biomechanics of tendon, followed by a description of the state of the art in understanding cell mechanical interactions with the extracellular matrix, and concluding with a perspective on the coupled, multiscale modeling required for understanding disease and proposing new therapies.

2 Tissue Structure and Biomechanics

Tendon is a composite material with an exquisite structure that lends the tissue excellent mechanical properties. Collagen, the most abundant protein in the tissue (80 % dry mass (Amiel et al. 1984) forms the basis for its hierarchical, multiscale arrangement.

Rod-like tropocollagen molecules ∼300 nm long and 1.5 nm diameter (Petruska and Hodge 1964) pack together in a staggered fashion with an overlap length of 67 nm, the so-called D-period (Hodge and Petruska 1963), providing a banding pattern visible in electron micrographs (Fig. 2). A microfibril, with five staggered tropocollagen molecules stabilized by covalent crosslinks, is the crystallographic unit cell and has a diameter of ∼4 nm (Orgel et al. 2006). Microfibrils interdigitate together to form collagen fibrils, and fibril diameter distributions vary according to

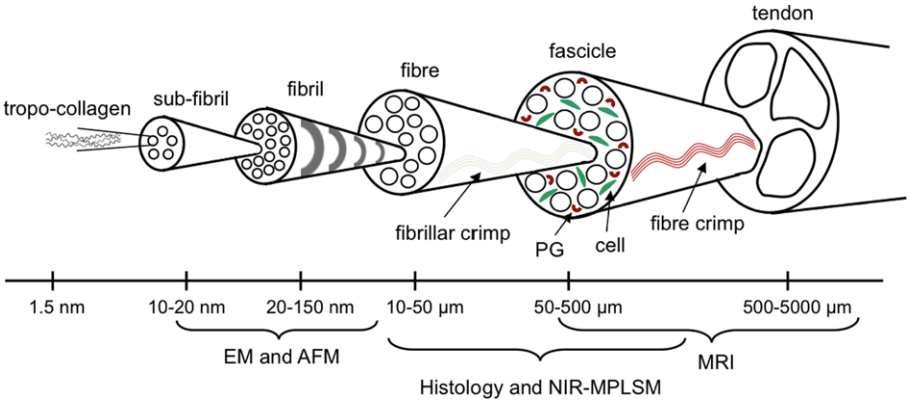

Fig. 2 Hierarchical structure of tendon from nanometer to centimeter scale with evidencing imaging modalities. Adapted from Baer et al. (1987)

age, tissue, and anatomical site with a maximum of ∼350 nm (Parry et al. 1978). The arrangement of microfibrils has a slight helicity to the axis of the fibril such that molecule ends appear on the surface of the bundle, allowing growth by accretion (Hulmes 2002). The fibril surface layer may have a different mechanical property to the interior, producing tube-like buckling and kinking behavior (Gutsmann et al. 2003) although an AFM scraping technique suggests a homogeneous rod structure (Wenger et al. 2008).

Fibril diameters increase with tissue maturity and the distribution of diameters reflects different tendon functional roles (Goh et al. 2012). An effect of fibril diameter distribution on tendon mechanical properties is expected due both to simple mixture theory and also through the specific surface area available for interface interactions with other matrix components (Goh et al. 2008), and fibrils of different diameters appear to experience similar in situ strains (Rigozzi et al. 2011). The length of fibrils increases with maturity, such that fibrils are continuous in the fully developed tissue as demonstrated by the lack of ends observed in electron microscope studies (Provenzano and Vanderby 2006). Further, theoretical estimates of lengths of the order of 10–100 mm have been made based on cell density and on the dependence of material properties on specimen length (Craig et al. 1989; Wang and Ker 1995; Legerlotz et al. 2010). The arrangement of fibrils is heterogeneous, with grouped bundles, plaits, and longitudinally aligned sections with fibrils crossing between, all observed in tendon (Provenzano and Vanderby 2006), and even interfibril fusion (Starborg et al. 2009). Locally, the major fibril direction is aligned with load bearing (Kannus 2000) with specialization at the enthesis or bone attachment (Benjamin et al. 2006).

Crimp is a microscopic wavy pattern in the collagen fibrils (Franchi et al. 2007) (Fig. 3). It has a repeat distance of the order of 0.1 mm (Grytz and Meschke 2009), possibly related to fibril tube buckling (Franchi et al. 2008), which potentially takes place due to the contractile action of tenocytes during fibril formation (Herchenhan et al. 2011). Tissue crimp is apparently the result of the alignment of ultrastructural sharp bends or knots of individual fibrils (Franchi et al. 2010). This pattern disappears

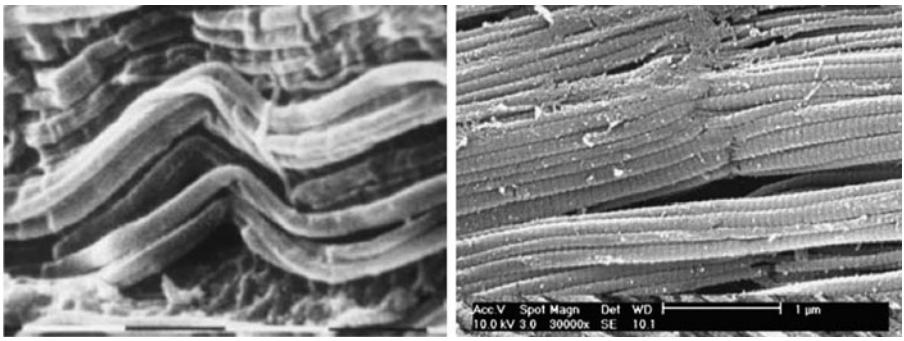

Fig. 3 Fibrillar crimp in relaxed and strained tendon, scale bar 1 µm. With permission from Franchi et al. (2007)

from optical microscopy and optical computed tomography under low strain (Franchi et al. 2007; Hansen et al. 2002; Boorman et al. 2006), and AFM of specimens fixed with glutaraldehyde suggested that fibril kinetics affect properties up to 5 % strain (Rigozzi et al. 2011). Fibrils are grouped together in larger and less well-defined fiber structures, and a bundle of fibers may make up a fascicle, a subunit of a tendon, but there is considerable heterogeneity according to tendon type and site (Kannus 2000).

Proteoglycans are present in tendon (∼1 % dry mass Rumian et al. 2007; Koob and Vogel 1987) consisting of a protein core and one or more covalently attached long chain polysaccharides, known as glycosaminoglycans (GAGs) (Yoon and Halper 2005). Small proteoglycans with low (<4) numbers of GAG chains appear to fulfil functions in controlling collagen fibril assembly and alignment, most prominently the molecule decorin binds to D-period spaced sites on collagen fibril surfaces (Parkinson et al. 2011). Genetically, modified knock-out mice, however, show that its absence may be compensated for in different anatomical sites by the action of other molecules during matrix assembly resulting in limited effects on material properties (Robinson et al. 2005). Knock-out of another proteoglycan, lubricin, known for its role in cartilage lubrication, did not change moduli, but significantly reduced the relaxation ratio of tail tendon fascicles (Reuvers et al. 2011). Although supposed mechanical interaction between collagen fibrils and large proteoglycans has been observed in electron microscopy of strained tissue (Cribb and Scott 1995), enzymatic removal produced similar strain responses to untreated controls (Lujan et al. 2009; Screen et al. 2005; Fessel and Snedeker 2011; Svensson et al. 2011). However, tissue hydration, which is provided by the hydrophilic nature of the large proteoglycans, has a strong effect on tissue mechanical properties (Screen et al. 2006), and stress driven interdiffusion of water between proteoglycan gel and collagen fibrils provides an important mechanism for deformation (Screen et al. 2011). Further, proteoglycan concentration varies within and between tendons according to local loading characteristics (Koob and Vogel 1987; Birch 2007).

Elastic fibers, composed of an elastin core surrounded by microfibrils, are present in tendon at levels between 0.1 % and 2 % dry mass and play a role in the tissue low

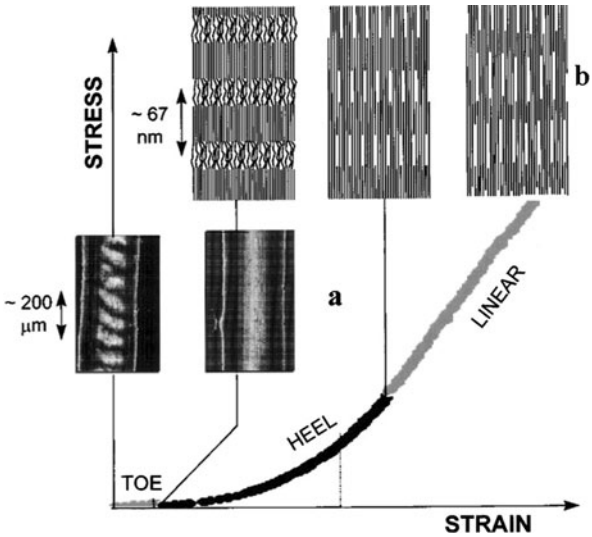

Fig. 4 Nonlinear stress-strain characteristic of tendon, with (**a**) polarized light microscopy showing loss of crimp and (**b**) straightening of molecular kinks followed by molecular gliding. With permission from Fratzl et al. (1998)

strain and resilience properties (Kannus 2000; Vogel 1991). Electron microscopy and immunohistology have shown elastic fibers bridging between collagen fiber bundles with a network structure (Parry and Craig 1978; Smith et al. 2011) and these are most abundant at sites close to bone attachment (Ritty et al. 2002).

The low stress-strain response of tendon is governed by the uncrimping of the collagen fibrils in the "toe" region (Diamant et al. 1972), followed by a straightening at molecular level in the "heel", and then, in the linear region, extension of fibrils (Fig. 4) (Fratzl et al. 1998). However, extension of collagen fibrils accounts for only 40 % of the tissue level strain in the linear region (Fratzl et al. 1998), with sliding at fibril and at fiber level observed both in vitro and in vivo apparently accounting for the remaining deformation (Cheng and Screen 2007; Snedeker et al. 2009). A model of stiff, high aspect ratio collagen fibrils embedded in a viscous proteoglycan matrix, the "ground substance," has been proposed (Puxkandl et al. 2002), with discrete fibrils effectively loaded in series with the matrix. However, as noted previously, fibrils in mature tendon may effectively be continuous along the length of the organ, with minimal requirement or benefit from load transfer across the matrix (Provenzano and Vanderby 2006). *Highly sparse* fibril branching may link all fibrils together in a continuous network (Starborg et al. 2009). Pull out tests of individual fibrils from rat tail tendons appear to show bonding to the surrounding tissue at regular D-period intervals (Gutsmann et al. 2004). At higher hierarchical levels, tendon fascicles are apparently able to slide independently of each other under axial load, with negligible lateral force transmission (Haraldsson et al. 2008).

The material properties of the tissue at each hierarchical scale are summarized in Table 1, showing decrease in Young's modulus with increase in scale, likely introducing gripping and size effects (Anssari-Benam et al. 2012) as well as the high variability inherent in testing biological material.

The sparse cell population in tendon (cell mass is only 1–3 wt% Jozsa and Kannus 1997) is located in distinct "tracks" between bundles of collagen fibrils with

Table 1 Multiscale properties of tendon, from ultrastructural to macroscopic. The quoted values of E, the Young's modulus, refer to the linear section of the stress-strain curve. The asterisked entry reports observed characteristic microfailure lengths rather than modulus

Structure	Technique	Modulus (E)
Molecule		
hydrated rat tail	Brillouin scattering	5.1–9 GPa (Harley et al. 1977; Cusack and Miller 1979)
hydrated fibroblastic human procollagen I	Single molecule optical tweezers and interferometry	0.35–12. 2 GPa (Sun et al. 2002)
hydrated bovine Achilles	X-ray diffraction	2.9 ± 0.1 GPa (Sasaki and Odajima 1996a)
Fibril		
hydrated bovine Achilles	AFM tensile force spectroscopy	0.2–0.8 GPa (van der Rijt et al. 2006)
dehydrated rat tail	AFM indentation	4–11.5 GPa (Wenger et al. 2007)
hydrated bovine Achilles	X-ray diffraction	0.4 GPa (Sasaki and Odajima 1996b)
*dehydrated rat tail	AFM force spectroscopy—pull-out of fibrils	22 nm and 78 nm characteristic length (Gutsmann et al. 2004)
hydrated human patellar tendon fibrils	AFM tensile test, direct comparison with whole tendon see below	2.8 ± 0.3 GPa (Svensson et al. 2012)
Fascicle		
rat tail	Tensile test (end effects corrected)	1.000 ± 0.165 GPa (Legerlotz et al. 2010)
bovine extensor	Tensile test (end effects corrected)	0.714 ± 0.120 GPa (Legerlotz et al. 2010)
rat tail	Tensile test (grip to grip)	329 ± 62 MPa (Hansen et al. 2009), 641 ± 30 MPa (Haraldsson et al. 2009) 668 ± 219 MPa (Fessel and Snedeker 2011)
human patellar	Tensile test, local strain	583–1231 MPa (Haraldsson et al. 2005)
human patellar	Tensile test, grip to grip	540 ± 230 MPa (Svensson et al. 2011)
human Achilles	Tensile test, local strain in PBS	226 ± 179 MPa (Komolafe and Doehring 2010)
Tendon		
human patellar	In vivo—torque and ultrasound displacement	0.8–1.1 GPa (Onambélé et al. 2007) 1.6–1.9 GPa (O'Brien et al. 2010)
human gastrocnemius	In vivo—torque and ultrasound displacement	250–300 MPa (Reeves et al. 2005)
human patellar	In vivo—torque and ultrasound displacement—direct comparison with fibril see above	2.0 ± 0.5 GPa (Svensson et al. 2012)

elongated mature cells, or tenocytes, of 20–70 μm length and 8–20 μm width approximately aligned with fibril direction (Kannus 2000). Immature tenocytes, known as tenoblasts, have a higher metabolic activity and are responsible for biosynthesis and secretion of ECM (Kannus 2000). Scleraxis (Schweitzer et al. 2001) is accepted as a genetic marker for the tendon phenotype; however, a recent study found that this gene alone is insufficient to discriminate tenogenic differentiation (Taylor et al. 2009). Tendon cells demonstrate tissue-wide signalling networks via connexins 32 and 43 parallel and perpendicular to fiber direction (McNeilly et al. 1996), which have been demonstrated to be involved in enhancing cell population response to mechanical stimuli (Waggett et al. 2006; Wall and Banes 2005). Cartilage studies have pointed out knowledge of the pericellular matrix (PCM) as essential for understanding tissue mechanobiology (Guilak et al. 2006), but little is known about this region in tendon. Loss of intimate contact between the cell and PCM was identified in tendons following unloaded culture, an effect rescued by inhibition of MMP activity (Arnoczky et al. 2007b), and microfibril proteins fibrillin and elastin appear to be colocalized in the pericellular region in tendon and ligament PCM (Smith et al. 2011; Ritty et al. 2002; Grant et al. 2013).

3 Cell Structure and Biomechanics

Cells have a dynamic internal structure, the cytoskeleton that mechanically links cell attachments to the extra cellular matrix to important organelles at a distance across the cell (Hu et al. 2003). The cytoskeleton is composed of polymers of actin, tubulin, and vimentin arranged in a network, apparently preloaded, reminiscent of "tensegrity" architectural and sculptural structures (Ingber 1993). Tubulin is observed to buckle with similar wavelengths in cells at rest, under externally applied loads and in areas with high actin concentrations in motile cells, with critical Euler loads of ~100 pN (Brangwynne et al. 2006). Tension applied by optical tweezers to actin fibers appeared to link directly to mechanosensitive ion channels providing an influx of Ca^{2+} ions into cells (Hayakawa et al. 2008), and use of actin polymerization disruptors such as cytochalasin demonstrate that a number of mechanosensing systems in cultured cells and tissue, including tendon, rely on the actin cytoskeleton for their mediation (Lavagnino et al. 2003; Myers et al. 2007; Marenzana et al. 2006; Koob et al. 1992; Arnoczky et al. 2004; Pavalko et al. 1998). In tendons, the actin cytoskeleton appears to help preserve cell–cell contact at gap junctions during tissue deformation (Wall et al. 2007a), is ideally placed for transducing tissue mechanical signals, and may provide an active mechanism for tissue recoil (Ralphs et al. 2002). The primary cilium, a hair-like structure known as a mechanotransducer and connected across the cell membrane to the cytoskeleton (Singla and Reiter 2006), is present in tendon cells and aligns with the local collagen orientation direction (Donnelly et al. 2010). The cell is anchored to the extracellular matrix at focal adhesions, where cell membrane proteins called integrins play a key role in transmitting load to the cytoskeleton (Wall et al. 2007b).

Mechanical models of cell behavior fall into two broad categories (Stamenovic 2008): (i) the tensegrity models, accounting for the role of contractile cytoskeleton

stress such as in durotaxis, the migration of cells toward stiffer substrates (Lazopoulos and Stamenovic 2008) or their linear stiffening under tensile load (Volokh et al. 2000), and (ii) the soft glass rheology model, accounting for both local and whole cell power law viscoelasticity (Fabry et al. 2001). The primary cilium, an organelle composed of tubulin with close association to the cytoskeleton, behaves as a heavy elastica under fluid shear load (Schwartz et al. 1997), and computational fluid dynamics confirms that the structure is highly sensitive to small shear stress loading (Chen et al. 2009).

The cytoskeleton is required for the movement of cells and enables them to apply traction to their surroundings and sense and respond to mechanical compliance (Lo et al. 2000), as well as bending local collagen fibrils and contracting the extracellular matrix across larger distances (Marenzana et al. 2006; Meshel et al. 2005). The role of the actin cytoskeleton in maintaining a residual tissue strain under zero external load through cell rearrangement of local collagen crimp has been demonstrated in embryonic periosteum (Foolen et al. 2010), and tendon cells appear capable of introducing crimp through cytoskeletal action on initially straight collagen fibrils in cultured "tendon-like" tissue constructs (Herchenhan et al. 2011).

Tendon cells are responsible for the deposition and remodeling of the collagenous extracellular matrix, and serial TEM sections of cultured cells have demonstrated specialized cell membrane extensions known as fibripositors assembling and extruding collagen fibrils (Canty and Kadler 2005).

A population of tendon stem/progenitor cells has been identified in tendon, capable of in vitro expansion and in vivo regeneration of tendon (Bi et al. 2007). This population proliferates and increases overall synthesis of extracellular matrix proteins in tendon in response to exercise (Zhang et al. 2010).

Tendon cells' in vitro responses to mechanical stimuli have been studied using silicone substrates applying simultaneous substrate deformation and fluid flow (Thompson et al. 2011), with low strain magnitude effects promoting anabolic activity, that is secretion of extracellular matrix proteins, and higher magnitudes more catabolic, that is, secretion of enzymes responsible for breaking down extracellular matrix proteins (Yang and Im 2005; Archambault et al. 2002; Yang et al. 2004).

4 Mechanical Tissue Models

Predicting tissue deformation, and hence mechanical stimuli at the cell scale is key to predicting cell, and hence tissue mechanobiological response. With the many different hierarchical structures in tendon, this requires challenging multiscale modeling to link macroscopic tissue deformation to cell response. The many phenomenological models of tendon deformation, based on parameters that have no direct basis in the microstructure of the tissue, are therefore not relevant for this review.

Molecular Dynamics (MD) techniques have successfully represented single collagen molecules and their assembly into a microfibril structure, predicting low and high strain moduli (300 MPa and 1.2 GPa) comparing well with experimental data (Gautieri et al. 2011). Such methods are computationally intensive and have not yet been employed for whole fibril simulations.

Models of fibril behavior have focused on representing the effect of crimp on the stress strain behavior of the tissue. Planar elastica theory identified that

smooth crimp shapes of continuous fibrils were consistent with tissue experimental stress strain curves (Buckley et al. 1980). A homogenisation of an elastica model to obtain a continuum strain energy function (Garikipati et al. 2008) also fitted tissue property data well up to strains of 20 %, and 3D helical fibril models required more parameters to achieve a similar fit (Grytz and Meschke 2009; Freed and Doehring 2005). All these studies neglect other tissue deformation mechanisms operating at strains above 2 % (Fratzl et al. 1998). Hinging fibers with zigzag geometries (Diamant et al. 1972; Stouffer et al. 1985) appear to reproduce optical microscopy observations more closely as well as the stress strain curve shape. Models representing the sequential recruitment of fibers successfully fit the nonlinear stress strain curve, but leave the mechanisms for recruitment open (Kwan and Woo 1989; Frisén et al. 1969).

Theoretical biochemical models of interactions effectively cross-linking collagen fibrils (Scott 2003) are included as a central feature of several Finite Element (FE) models (Puxkandl et al. 2002; Ciarletta et al. 2006). However, extensive empirical evidence cited previously and coupled FE modeling appear to rule out a direct contribution of such cross-links to tensile deformation behavior (Fessel and Snedeker 2011).

One outstanding multiscale model of whole tendon tissue includes both fibril and fibril bundle behavior (Hurschler et al. 1997). The alignment of fibrils, embedded in an incompressible matrix supporting no shear stress, and the straightening of fibers (bundles of fibrils) are modeled by probability density functions, alongside stretch-based failure criteria at both fibril and fiber level. The seven parameter model gives a good fit to selected experimental data and illustrates how a homogenized model can provide insight into microstructural deformation. Homogenization methods are also used to predict the tissue's large Poisson ratio (Reese et al. 2010), which is of direct relevance to mechanical signals perceived by cells.

5 Mechanobiology of Tendon

The sensitivity of tendon in health and disease to its mechanical environment is well documented in animal and human studies. A review of human studies identified that tendon material properties decline with age, an effect which can be mitigated by training, though chronic unloading of tendon caused atrophy as well as loss of material properties (Reeves 2006). Comparison of tendons across anatomical locations suggests that they are adapted for fatigue performance at the stress they most frequently experience during everyday life (Ker et al. 2000), so adaptation following training may also target fatigue properties. *Studies* comparing low "stress-in-life" positional tendons with high "stress-in-life" energy storing tendons in horses, however, found higher matrix turnover in the positional than in the more critically loaded energy storing tendons (Birch et al. 2008; Thorpe et al. 2010). Training, with appropriate rest periods, increases collagen metabolism and boosts anabolic processes that build up proteins and extracellular matrix. Catabolic processes that break down proteins enzymatically are increased also but to a lesser extent, so the balance results in tendon hypertrophy (Magnusson et al. 2010). Tissue fatigue damage, with fibrils kinking then

failing, accumulates as a result of cyclic loading, leading surprisingly at low levels to slight tissue stiffening, and then to progressive loss of stiffness (Fung et al. 2009; Fung et al. 2010). The boundaries between stimulus levels that provoke overall tissue anabolic effects, promoting repair and strengthening and overall catabolic effects, potentially leading to disease, are narrow and their position uncertain (Arnoczky et al. 2007a). Tendinopathy, a degenerative disorder of tendon, shows distinct loss of material properties, at the same time as increases in cross sectional area (Arya and Kulig 2010), and appears to result from an imbalance in the anabolic and catabolic responses to loading (Magnusson et al. 2010).

Computational modeling has been directed at the understanding of the mechanobiological response of bone during development, remodeling and healing for several decades (Carter and Wong 1988; Carter et al. 1998; Lacroix and Prendergast 2002; Isaksson et al. 2006). These models use iterative updates to the tissue distribution in a finite element model driven by local continuum measures of tissue deformation. It is well accepted that such models are able to represent important stages in these biological processes and can assist in the hypothesis driven investigation of novel bone regeneration therapies (Lacroix et al. 2009). Tendon tissue has not featured within these models, but appropriate data both from histological studies of tendon healing under controlled mechanical conditions in animals (Eliasson et al. 2009; Virchenko et al. 2008) and from investigations of the mechanical properties of healing tendon in patients (Schepull et al. 2007; Brown et al. 2012) are available.

Such mechanobiological models might start from the basis of a multiscale finite element representation of cells embedded in a poroelastic, fiber reinforced matrix. Using this model, fluid flow over the cell membrane, rather than cell membrane strain, was identified as the likely stimulus for observed cell down-regulation of matrix degrading enzymes (Lavagnino et al. 2008). This assumption is strengthened by recent observations showing that tendon cell cilia deflect in response to tissue loading (Lavagnino et al. 2011). However, a role for strain-based cell—tissue interaction clearly must be allowed in such models, with cell contractile behaviour important in setting up crimping in collagen fibrils (Herchenhan et al. 2011). Computational mechanobiological models have successfully predicted changes in collagen orientation in response to mechanical signals in blood vessels (Hariton et al. 2007). In bone healing, multiscale modeling is able to take into account patterns of cell differentiation in response to local fluid flow, and hence predict mineralization patterns in response to different levels of mechanical stimulation (Vetter et al. 2012). Multiscale modeling of fibroblasts and growth factor diffusion through extracellular matrix proteins at the site of an injury, has provided a prediction tool for scar tissue organization (McDougall et al. 2006). A predictive model of tendon healing would combine these features and enable for example prediction of the major factors affecting time to healing following an Achilles rupture.

6 Future Modeling Developments

In order to realize the enormous potential of mathematical modelling to contribute to the design and development of tissue engineering and regenerative approaches for tendon progress toward the following three linked challenges to be made:

(i) Models for cell anabolic and catabolic processes, especially of collagen, are required, predicting quantities and orientation of the fibrils secreted, as well as quantities of matrix proteinases. This modeling needs to proceed in parallel with experimental observation of these processes.
(ii) Micromechanical models of the cell local environment are required that include the specialized pericellular and local microstructure of the extracellular matrix. These should be capable of predicting shear stresses at the cell membrane due both to interstitial flow, but also due to the extensive relative sliding of the fibrils to which individual cells are attached. Incorporation of coupled models of cell response will enable prediction of ECM changes due to mechanical stimulation.
(iii) Finally the challenge of linking micromechanical models to macroscopic deformation of particular tendons must be addressed. This requires resolution of several outstanding questions on the organization of ECM in tendon, including the length and connectivity of fibrils and the role of glycosaminoglycans. Further, the macroscopic geometry of the tendon needs to be captured and the effects of nonhomogeneities, including varying water content and concentrations of ECM proteins should be included.

Mathematical models, informing and informed by experimental models, will form a vital part of the approach to these challenges.

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