A conceptual framework for computational models of Achilles tendon homeostasis

David W. Smith,1∗ Jonas Rubenson,2 David Lloyd,3 Minghao Zheng,4 Justin Fernandez,5 Thor Besier,5 Jiake Xu6 and Bruce S. Gardiner1

Computational modeling of tendon lags the development of computational models for other tissues. A major bottleneck in the development of realistic computational models for Achilles tendon is the absence of detailed conceptual and theoretical models as to how the tissue actually functions. Without the conceptual models to provide a theoretical framework to guide the development and integration of multiscale computational models, modeling of the Achilles tendon to date has tended to be piecemeal and focused on specific mechanical or biochemical issues. In this paper, we present a new conceptual model of Achilles tendon tissue homeostasis, and discuss this model in terms of existing computational models of tendon. This approach has the benefits of structuring the research on relevant computational modeling to date, while allowing us to identify new computational models requiring development. The critically important functional issue for tendon is that it is continually damaged during use and so has to be repaired. From this follows the centrally important issue of homeostasis of the load carrying collagen fibrils within the collagen fibers of the Achilles tendon. Collagen fibrils may be damaged mechanically—by loading, or damaged biochemically—by proteases. Upon reviewing existing computational models within this conceptual framework of the Achilles tendon structure and function, we demonstrate that a great deal of theoretical and experimental research remains to be done before there are reliably predictive multiscale computational model of Achilles tendon in health and disease. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

Tendons transmit tensile forces between muscle and bone and endure repeated loading as muscle contracts. For human Achilles tendon, the forces transmitted are a large fraction of the ultimate load carrying capacity of the tendon.1 One advantage of high working strains is the reduction in the mass of the Achilles tendon, which reduces the inertia, and thus the mechanical work of swinging while walking or running. More importantly, high working stresses facilitate the storage of considerable potential strain energy as the Achilles tendon is stretched. This recoverable strain energy reduces the energy of locomotion by reducing the mechanical work required of the muscle.2,3 Indeed, it has been shown that small changes in the length of the calcaneous
significantly influence the strain energy stored in the Achilles tendon, and so running economy. Running economy and efficiency may be quantitatively assessed using computational models of the muscle-tendon unit. These computational models typically include, with varying degrees of sophistication, the potential energy storage by the tendon and force generation by the muscle. The models have shown that efficiency gains in the muscle-tendon unit result from changes in tendon length by enabling muscle fibers to maintain a favorable velocity for force generation and power outputs, while simultaneously storing strain energy that is recovered later in the gait cycle.

While a key advantage of high working strains in the Achilles tendon is improved running economy, the disadvantage of high working strains is the damage this inflicts on the tendon. Persistence hunting, an advantageous ecological niche for early humans, requires endurance running. Endurance running necessarily involves many thousands of repeated loadings of the Achilles tendon. Repetitive loadings at high working strains lead to so-called fatigue damage of the tendon. Tendon damage increases as the maximum strain increases and as the number of loadings increases. The combination of high strain and high number of repeated loadings means that the Achilles tendon is at risk of ‘over-use injury’ and possible rupture. Tendon rupture is almost always preceded by tendon degeneration.

However, the probability of tendon failure during the next period of exercise is reduced if the tendon can be effectively repaired. Indeed, if the rate of tendon repair matches the rate of tendon damage, then tissue homeostasis and function are maintained indefinitely. It is likely that derangements of tissue homeostasis leads to non-inflammatory tendonosis, and ultimately to tendon rupture. A detailed, quantitative understanding of tissue homeostasis is of critical importance in developing effective strategies for occupational and sports management, and for effective therapeutic interventions, surgical or otherwise. To this end, this paper develops a conceptual framework that will enable the future development of reliably predictive multiscale computational models of Achilles tendon in health and disease. As we develop our conceptual model we review the existing literature on computational models relating to the steady-state maintenance (or homeostasis) of the Achilles tendon. It is our view that the relatively small number of models of tendon homeostasis in the literature (compared with other tissues) reflects a need for more clearly elucidated conceptual models of how this tissue works to maintain its functional mechanical properties despite incurring damage during daily activity. Herein we attempt to provide the key concepts and organizing principles for future computational model development for Achilles tendon.

**MODELING ACHILLES TENDON STRUCTURE**

Form follows functions, and so elucidating tendon structure is of critical importance to a detailed understanding of how a tissue maintains itself. Further, the structure of tendon is important when considering how to construct realistic computational models of the tissue system. For example, a hierarchical spatial arrangement of the tissue itself may suggest a logical structure for the computational model based on the various processes occurring at different length scales. For example, length scales within a tissue define the timescales for mass transport, which may become a rate-limiting step for a tissue repair process. While considering structure we also seek to identify any distinct compartments within the tendon, because tissue compartments are usually present in biological systems to facilitate and regulate tissue processes, both during development and for maintaining tissue homeostasis. For all these reasons, a clear understanding of the micro-scale and macro-scale structure of the tendon is a central concern when developing realistic computational models of tendon.

The structure of tendon is described by various authors using different nomenclatures. Here we adopt the nomenclature of Kannus as shown in Figure 1.

The human Achilles tendon is clearly hierarchical in its structural organization (Figure 1), suggesting a natural organization for both theoretical and computational models capturing the various processes occurring at each length scale. We note that, to date, there are no comprehensive multiscale models of tendon motivated by its structure, although we shall see that considerable effort has been expended in developing models that span one or more length scales.

Functionally, a crucially important length scale occurs at the level of the collagen fiber (Figures 1 and 2). A collagen fiber is made up of many collagen fibrils, separated by an extracellular matrix that is composed primarily of proteoglycans (such as versican and aggrecan). Jarvinen et al. found that the thickest collagen fibers in humans are found in the Achilles tendon, and that their average diameter is about 28 μm. Significantly, these authors found that in ruptured tendons the average diameter of collagen fiber is smaller relative to controls (about 18 μm).

Moving down a length scale from the collagen fiber is the collagen fibril (Figure 1). On average,
FIGURE 1 | The organization of tendon structure from collagen fibrils to the entire tendon. Note that collagen fibrils are comprised of polymerized tropocollagen mers.\textsuperscript{24,25}

FIGURE 2 | Confocal microscope image of a fluorescein-stained surface from a mature New Zealand white rabbit Achilles tendon, which has been cut through with a sharp scalpel and imaged. White arrows show collagen fibers, while the faintly visible lines within the collagen fibers are comprised of groups of collagen fibrils. Image size: width and height 750 \( \mu \text{m} \). (Confocal image by Dr. Ping Wu)

there are several thousand fibrils in a large collagen fiber. Kannus found the fibril diameter in the Achilles tendon to be in the range 30 and 130 nm (with most between 50 and 90 nm), while Magnusson et al. found Achilles tendon fibrils in the range 90–150 nm. Magnusson et al. also reported far fewer fibrils in some parts of ruptured tendons, which appears to be consistent with the findings of Jarvinen et al.,\textsuperscript{27} who reported thinner collagen fibers in ruptured tendons. It is noted that collagen fibers in tendon are essentially unimodal in their principal directional fiber orientation, but sheets of collagen are often bimodal in their principal fiber directions. Schreifl et al.\textsuperscript{28} describe an automated method for characterizing the principal collagen fiber orientations from two-dimensional images, together with the collagen fiber dispersion as well as changes in fiber distributions at different length scales.

The length of collagen fibrils is uncertain. Some authors argue that in a composite of collagen and proteoglycan matrix, the collagen fibrils have no need to span the length of the tendon. Rather, the only requirement is that the fibril length must exceed the theoretical critical length for composite fiber-reinforced materials (estimated to be in range of 30–100 \( \mu \text{m} \) for tendon).\textsuperscript{29} For the rat-tail (which is 35 to 50 mm long) some researchers have obtained estimates of fibril lengths varying from 0.5 to 5.9 mm or 0.31 and 12.7 mm.\textsuperscript{30} It is possible that collagen fibrils either end, branch, or are joined together via ‘linker molecules’ to span the length of the collagen fiber. Redaelli et al.\textsuperscript{30} have accepted this composite linker-molecule model of tendon structure and computationally investigated many different geometric configurations of collagen fibrils linked together by decorin proteoglycans. They have used their computational model to estimate the micromechanical properties of these collagen proteoglycan aggregates.\textsuperscript{30}

However, Provenzano and Vanderby\textsuperscript{31} make a persuasive argument for collagen fibril structure changing with the developmental stage of the animal,
and found that for tendon in mature animals the collagen fibrils run the full length of the tendon. They say:

Hence, in mature tendons and ligaments we conclude that collagen fibrils are very long, likely spanning the length of the tissue with a mean fibril length equal to the tissue length or greater since fibril do not lie in straight lines origin to insertion. This conclusion, supports the concept that force in these tissues is transferred directly through long fibrils. In regard to the theory that force transmission in ligament and tendon occurs via short collagen fibrils connected by load transferring proteoglycans, we feel that this theory is not supported.

It is noted that Svensson et al. have reported on the mechanical properties of primary fibers and individual fibrils, loaded at different rates. The fibrils themselves are composed of many tropocollagen units. Each tropocollagen unit is approximately 1.5 nm in diameter and 300 nm long. The tropocollagen molecule consists primarily of a left-handed triple helix of amino acids. Based on minimization of chemical potential energy, Israelowitz et al. successfully modeled the formation of the tropocollagen triple helix.

Sophisticated molecular dynamics (MD) simulations of the force-extension characteristics of individual tropocollagen units now exist. For example, in’t Veld and Stevens found that upon loading the triple helix first unwinds, then straightens, which leads to a bilinear force-displacement curve. Parkinson et al. developed a computational model of tropocollagen unit assembly into collagen fibrils, using up to 50,000 rod-like particles of different configurations, and also examined the mechanical properties of the aggregates. More sophisticated models of collagen fibrils and other elastomeric proteins have been developed to model ‘aggregate assembly’, meaning collagen fibril formation.

Moving up a length scale from the collagen fibers are the ‘primary fibers’ of the tendon (Figure 1). The primary fibers typically have diameters in the range of 15–400 μm (with diameters in the upper range being found in large tendons like the Achilles). The length scale for the maximum diameter is significant because 200 μm (i.e., half the maximum diameter) is about the maximum distance a cell (e.g., a tenocyte) can be from a capillary, if it is to maintain adequate oxygen and nutrition and remove its waste products effectively. The authors are unaware of any computational models attempting to analyze the oxygenation and nutritional status of tenocytes in primary fibers.

Indeed, blood vessels and nerves are found in the loose connective tissue known as endotendon located around primary, secondary, and tertiary fibers (Figure 1). The blood vessel size presumably increases with the thicknesses of the fascicle and endotendon sheath. It has been shown that blood vessels may enter the ends of tendon and travel longitudinally along the long axis of the Achilles tendon, but importantly there are significant lateral anastomosis between blood vessels traversing the paratendon and the highly vascularized epitendon (outer most covering of the tendon). The mid-section of the Achilles tendon is reported to be hypovascular, while the paratendon functions as a sleeve permitting free movement of the tendon next to surrounding tissues. Although likely to be functionally important, the authors are unaware of any computational models describing mechanical stretching and its effect on the vascular connections, either within the tendon or between the paratendon and the epitendon.

Importantly, the paratendon creates two compartments: the compartment inside containing the tendon itself and the compartment outside containing the surrounding tissue. In this context it is noted that microdialysis of the fluid within the peritendinous sheath around the Achilles tendons has revealed very high levels of collagen synthesis (estimated to be about 1% of the total collagen mass per day) by cells that can supply synthetic products to this compartment. It is likely that collagen mers diffuse preferentially along the endotendons (which themselves are highly cellular), perhaps helping to supply collagen mers for new collagen fibril and fiber formation within tendons. This implies that a substantial blood supply to the paratendon is required to support the high collagen mer synthesis rate, which is likely to be necessary but not sufficient condition to avoid tendinopathy. The authors are unaware of any computational models describing the movement of mers from the peritendinous space through the endotendon to deeper tendon tissue, or vice versa.

It is highly likely that the collagen fibers run the entire distance between the muscle and bone attachments but their arrangement may be different between species. For example on the basis of stress–strain curves for whole tendon and its constituent fascicles from the superficial digitorum flexor of the horse, there is a reason to question whether the tendon fascicles run the entire distance between muscle and bone in the horse. However, careful mechanical tests have shown that shear transfer between fascicles in the human Achilles tendon is negligible, and so fibers are likely to run the whole length of the tendon.

The individual collagen fibrils and fibers sometimes spiral around each other in a helix.
and the same may happen at the level of secondary and tertiary fibers. For example, at the whole tendon scale, the segment of the Achilles tendon attached to the soleus muscle spirals a quarter turn around that segment of the Achilles tendon attached to the gastrocnemius muscle. The authors are unaware of any macroscale computational models of tendon describing the effect of this quarter turn on tendon mechanics, but it is likely that spiralling helps prevent ‘bowstringing’ of the tendon fascicles. The need to resist bowstringing is likely to be substantial given the high in vivo transverse strain recently observed in both avian and human Achilles tendon, and may even act to modify the macro-scale longitudinal stiffness of the tendon.

Indeed at all length scales the spiralling of fibers or fascicles helps to draw the tendon together like a rope upon loading so avoiding bowstringing of the fibers, which may conceivably mechanical disaggregate the tendon into its constituent fascicles if unchecked. Importantly, the drawing together of the tendon components at different length scales results in fluid being squeezed from the tissue as it is tensioned. Mechanically squeezing and relaxation may assist in the distribution of molecules of all types both along and across the tendon. Indeed, given that mechanics is the tissue’s raison d’etre, it seems likely that mechanically assisted transport across and along the tendon could play an important role in maintaining a healthy tendon. In addition, it is likely that in vivo blood flow to the tendon is periodically interrupted as the tendon is cyclically loaded, much as it is in other cyclically loaded tissues, such as muscle. Currently, there are no models capturing the interactions between transport and mechanical loading, as have been developed for other tissues.

Importantly, McNeilly et al. and Ralphs identify another much smaller, tissue compartment within tendon. They report that collagen fibers are completely enclosed by a confluent sheet of tenocytes (Figure 3). This confluent sheet of tenocytes creates interior and exterior compartments. These compartments are almost certainly of considerable functional significance for collagen fibril homeostasis located within the collagen fibers. Ralphs describes the arrangement of tenocytes around a collagen fiber:

Tendon cells are arranged in longitudinal rows between collagen fibre bundles. Within rows, they are in longitudinal contact end-to-end; between rows they are in contact via sheet-like lateral cell processes that extend around collagen fibre bundles and meet up with processes from adjacent cells. An individual cell is associated with 6–9 collagen fibre bundles, and

**FIGURE 3** | Diagram to illustrate interaction of cells and association with collagen fiber bundles (cylinders); two cells are shown longitudinally (lower cells), and two laterally. Collagen bundles are enclosed by lateral cell processes and passed from cell to cell longitudinally.

Tenocytes communicate with their neighbors through several different types of connexin junctions, which permit the free movement of certain kinds of molecules between the cells. It is probable that connexin 32 promotes matrix synthesis whereas connexin 43 blocks it. Recently it has been shown that mechanical loading influences the number of connections between tenocytes. Tenocytes almost certainly adjust their shape depending on their function. Tenocytes are usually spindle shaped, but may become rounder when required to increase their rate of synthesis significantly, or they may appear chondrocyte-like when subject to compressive forces, as in regions where the tendon wraps around a bone (Figure 4).

In other words, the tenocytes adapt, as the tendon needs to respond to different environmental conditions and/or stressors, including injury. Provenzano et al. define a grading scale for connective tissue injury (Grade I injury is a mild stretch with no discontinuity, Grade II is moderate stretch with some collagen fibers torn, while Grade III is severe stretching with complete or nearly complete disruption and significant joint laxity). Crucially, they observe that Grades I and II ‘sub-failure’ damage is mended by the tenocytes (which are specialized fibroblasts) alone, through a non-inflammatory repair process, i.e., the repair process does not involve leukocytes:

Interestingly, this remodeling process appears intrinsic with little or no inflammation response as damaged
FIGURE 4 | Examples of the three morphological tenocyte types reported in equine digital tendons: (a) type 1 cells with long, spindle-shaped nuclei; (b) type 2 cells with plump, cigar-shaped nuclei; and (c) chondrocyte-like type 3 cells, typically found in ‘wrap around’ regions of tendons where the matrix is fibro-cartilaginous. Stain haematoxylin and eosin (magnification ×400).55

The tissues show no changes in macrophage or neutrophils levels following injury and expression of the inflammatory markers, tumor necrosis factor-α and tartrate-resistant acid phosphatase were unchanged. Hence, since inflammation plays a large role in wound healing by inducing cell migration and proliferation, and controlling extracellular matrix scar formation, its absence leaves fibroblasts to principally direct tissue remodeling. Therefore, following a Grade II subfailure injury to the collagen matrix, we conclude that tissue remodeling is fibroblast-mediated and occurs without scar tissue formation, but instead with type I collagen fibrilogenesis to repair the tissue. As such, this system provides unique insight into acute tissue damage and offers a potentially powerful model to examine fibroblast behavior.

When the tendon is acutely overstressed, the tenocytes may increase in number and change their shape, becoming more rounded.57 Cook and Purdam58 label this adaptive state ‘reactive tendinopathy’. In this state the tissue observations are increased amounts of mucoid ‘ground substance’ (i.e., principally proteoglycans) and ‘tendon thickening’ (which is probably due to the osmotic imbibition of water induced by the proteoglycans). With ongoing mechanical cyclic strain, this reactive tendinopathy may progress to ‘tendon disrepair’. Tendon disrepair is more focal and characterized by initial collagen fiber disorganization and chondrocytic and myofibroblastic cells producing greatly increased quantities of proteins and proteoglycans. Tendon disrepair may finally progress to ‘degenerative tendonopathy’. Degenerative tendonopathy is characterized by the hyalinization of the tendon and/or fibrosis, cell apoptosis, and acellularity. Various histological classification systems have been developed to characterize these changes.59

It is noted that normal cell ‘phenotype maps’ have been experimentally characterized, with cell phenotype driven by the environmental stress state and the fluid flow that the tissue experiences.60 However, despite cell populations models involving proliferation and differentiation and apoptosis being developed for other tissues,61,62 as far as we are aware to date there are no computational models describing changes in tendon cell number or their phenotypes.

Interestingly, temperatures as high as 41°C have been estimated at the center of the human Achilles tendon during cyclic loading, generated by the frictional heat as a result of load-cycle hysteresis.45 It has been postulated that this elevated temperature may contribute to an elevated rate of tenocyte apoptosis at the center of the tendon, which may lead to tendinosis. This brings us to the mechanical response of the tendon.

MODELING THE MECHANICAL RESPONSE TO LOAD AND TENDON DAMAGE

Achilles tendon is a compliant connector between muscle and bone, storing potential strain energy as it is subject to peak stresses in excess of 70 MPa in humans.12 Perhaps unsurprisingly, modeling the mechanical behavior of tendon has received more...
Mechanical modeling of tendon has been approached in a variety of ways. Macroscale continuum modeling approaches usually focus on ‘large deformation analysis’ to describe the strain. Model differences emerge at the level of the constitutive equations employed to describe the tendon load-deformation behavior. These constitutive equations generally vary macroscale parameters for characterizing anisotropy, incompressibility, bi-phasic (fluid and solid) constituents, damage evolution, and time-dependent behavior. Investigators have developed ‘composite solid’ models, viscoelastic models, poroelastic models, and hyperelastic models.

The viscoelastic behavior of tendon is at least partly explained by the movement of fluid within the tissue, so poro-viscoelastic models are probably more appropriate, but these types of models are yet to be developed for tendon. Tang et al. noted that viscoelastic models may be easily over-parameterized. A great deal of time and interest has been expended attempting to model the crimp in tendon, with a variety of approaches suggested (see for example Grytz and Meschke for the development of their ‘coiled spring model’).

Reese et al. developed a microscale finite element composite solid model, which consists of a collection of collagen fibrils explicitly modeled within a collagen fiber (Figure 5). They use an isotropic neo-Hookean hyperelastic constitutive equation to describe both the load-deformation behavior of the fibrils and the extracellular matrix between the fibrils. The authors perform numerical homogenization to find the estimates of macroscale parameters such as the Poisson’s ratio, which are then compared with experimental findings. They showed that a helical fibril organization within a crimped fiber was capable of predicting the nonlinear stress–strain behavior and the large Poisson’s ratio that are observed experimentally. They found the predicted macroscale Poisson’s ratio was strongly dependent on the helical pitch, crimp angle, and the material coefficients.

Reese et al. observed that stretched fibers are drawn together and ‘almost touch’, which in a poroelastic would drive the exudation of fluid from the tendon. As noted, it is likely that the cyclic movement of fluid in and out of tendon is an important contributor to the transport of nutrients, mers, growth factors, and signals of various kinds within the tendon. To date, there are no coupled deformation-transport computational models of mechanical and transport processes within the tendon.

A major limitation of all the foregoing computational models is that they do not include fiber damage, which requires the inclusion of an ‘irreversible’ damage process. Poro-visco-elastic–plastic models may be suitable as an initial damage model, but as far as the authors are aware to date none exist. For example, the model of Reese et al. includes elastic fiber extension, but does not include fiber damage.

The paper by Natali et al. develops a microscale model of collagen fibrils (linked by decorin), and embeds this within phenomenological hyperelastic constitutive equations representing both matrix and fibers (with adjustable crimp). Natali et al. also include a ‘damage function’ that depends on a normal distribution of fiber length and fiber
number. Using their multiscale model, they can reproduce the strain softening part of the loading curve arising as a result of progressive fiber damage experienced by the tissue.

Ciarletta et al.\textsuperscript{70} built a multiscale model similar to that of Natali et al., although Ciarletta et al. use a modified strain energy density function. The strain energy density function is multiplied by a continuous ‘softening function’, which represents ‘structural changes’ taking place in tendon upon loading, and a separate dissipation function is added to the strain energy density function. The structural changes represented in the model are fiber recruitment, and a thermally activated transition-state theory that accounts for ‘rupture or reformation of the GAG-collagen type I junctions’. We note here that ‘reformation’ of fibers relates to the computational modeling of repair processes within tendon. While the papers of Natali et al.\textsuperscript{69} and Ciarletta et al.\textsuperscript{70} represent important steps forward, the difficulty of the approaches is that the physical significance of many of the parameters in the sub-models employed in their continuum model are either obscure or not closely related to the known physiology, thereby limiting their application and interpretation.

On the other hand, the paper by Bontempi\textsuperscript{74} has the merit of being both comparatively simple and having parameters closely connected to the known physiology and physics of tendon. Bontempi outlines how a linear-load extension relationship for each individual collagen fiber together with fiber recruitment may explain the load-deformation curve for tendon. His explanation for the load-deformation curve is not incompatible with the observation that collagen crimp is highly variable over short distances in Achilles tendon 24. It is also clear that there are many different assumptions about the microscopic behavior of collagen fibrils that can accord with existing macroscopic observations on the load-extension behavior of tendon.

Importantly, Bontempi\textsuperscript{74} shows how a probabilistic damage function may be included in the analysis. He proposes that fibrils break with a certain probability dependent on their taught extension length. Breakage reduces the stiffness and the ultimate load carrying capacity of the tendon. This damage function may be calibrated with experiments based on experimentally observable changes in the load-deformation curve. It is noted in passing that with an appropriate initial distribution of collagen fiber lengths, it is likely that Bontempi’s model would be able to represent, for example, the experimental observations of Andarawis-Puri and Flatow’s,\textsuperscript{75} that is, ‘the unexpected increase in stiffness and decrease in hysteresis that was observed for low and moderate levels of fatigue loading is likely attributable to redistribution of loads from non-load bearing damaged fibers to undamaged fibers’.

Ideally, a continuous experimental damage function is needed that incorporates both the number of load cycles and strain extensions, although a series of discrete damage functions would be an important step forward. Unfortunately, much remains to be discovered about the centrally important fatigue properties of tendon.\textsuperscript{76,77} In this context, it is noted that in one experiment on humans,\textsuperscript{78} which centered on ‘exercise to exhaustion’ by a group of athletes, no statistically significant change in tendon stiffness was observed (suggesting no detectable tendon damage). However, it is likely the study was almost certainly underpowered.

Indeed, in an important study Onambele-Pearson and Pearson\textsuperscript{79} found that tendon stiffness decreased from morning to evening (consistent with fibril damage), and then the stiffness increased again between evening and the morning (consistent with fibril repair), establishing a diurnal pattern to tendon stiffness. These findings are clearly consistent with many experimental animal models clearly showing that fibril damage accumulates with load and strain\textsuperscript{76,80} as a result of daily activities. Fibril damage may be more subtle than outright rupture—initial damage may involve a combination of denaturation, fragmentation, stretching, splitting, and fraying of the collagen fibers and fibrils,\textsuperscript{27} progressing to outright rupture.\textsuperscript{76,80} A very useful and powerful conceptual model of collagen fibril damage is shown in Figure 6. It is noted that some damage-grading systems based on histological appearance appear to be consistent with the damage model as shown schematically in Figure 6.\textsuperscript{81}

What is clear from experiments to date is that mechanical damage of tendon is nonlinear, and that the damage becomes progressively more severe as fiber extension increases.\textsuperscript{56,76} Indeed, the animal study by Fung et al.\textsuperscript{76} demonstrates how increasing cyclic strain leads to a pre- and post-testing clamp-to-clamp strain increment, indicating increasing levels of fibril damage as the fiber length increases. It is noted that Wren et al.\textsuperscript{1} found that ‘initial strain’ (measured strain on first loading to a predetermined stress level) of Achilles tendon was one of the better predictors of fatigue life. It is also noted that an increasing tendon fiber length is detected histologically as an increasing ‘waviness’ of a sectioned tissue.\textsuperscript{57}

Proteases may degrade collagen fibrils and proteoglycans. Importantly, there are strong interactions between proteolytic degradation and mechanical
FIGURE 6 | Schematic diagram of damage mechanism that underlies tendon fatigue. Initial fatigue loading lengthens the fibers from the crimped (a) to the uncrimped (b) state. Continued loading causes stretching of a local population of fibers into their plastic range of deformation (c, dashed line), resulting in the formation of kinked fiber deformation patterns. Further loading leads to rupture of the plastically deformed fibers (d, dotted line). Subsequently, loading is assumed by the surviving, intact fibers with longer lengths and/or higher fatigue quality (dashed line).  

Activated proteases remove damaged or unwanted extracellular matrix as part of normal tissue turnover and collagen fibril homeostasis. For example, following mechanical fibril damage, debridement of the damaged fibril by proteases may be one of the early steps in fibril repair. Lauer-Fields et al. have measured the kinetic parameters for a number of metalloproteases, while Tzafriri et al. have developed a detailed computational model based on a reaction–diffusion analysis of fibril degradation. They confirmed that all the parameters in the model may be identified for an in vitro experimental system of sparse collagen fibrils. However, Tzafriri et al. model does not include the effect of regulatory collagens or proteoglycans (e.g., small leucine-rich proteoglycans—SLRPs), which are present in vivo and are likely to play an important role in regulating collagen fibril homeostasis. For example, decorin binding to collagen fibrils may both inhibit polymerization of more tropocollagen while simultaneously reducing or preventing proteolytic degradation.

But there is a second key interaction between mechanics and proteolytic degradation. Following on previous work, an important paper by Wyatt et al. shows how protease efficacy strongly depends on mechanical strain experienced by the collagen fibrils. They found that bacterial collagenase rates of degradation for a rat-tail model collagen decreased 73% as strain in the collagen increased from 1 to 4%, and by extrapolation, they estimated a zero rate of proteolytic degradation at about 6% strain. These observations suggest that any collagen experiencing strain above about 3–4% may be protected from degradation by collagenases. It is as yet not known if intermittent cyclic loading (as say, experienced by tendon in daily activities) is protective of proteolytic degradation, but based on the findings of Wyatt et al. it seems likely that it is.

A detailed understanding of the action of metalloproteases on collagen fibrils has recently become available. For example, Rosenblum et al. imaged a (MMP9) metalloprotease at work on (Type II) collagen using an atomic force microscope in tapping mode. They found that:

Initially, MMP-9 is randomly distributed along the collagen fragment, becoming localized at the tail as the reaction progresses. This may indicate that the collagen molecules become more relaxed with time promoting higher affinity to the enzyme, particularly at the tails. An alternative interpretation is that the enzyme inches along the collagen, scanning for denatured and damaged regions or its cleavage recognition sequence. The ability of MMPs to move...
along collagen fibrils has been investigated, and it is suggested that MMPs may utilize a Brownian ratchet mechanism for “biased diffusion”.

The process of ‘itching along the collagen’ by diffusion has been confirmed by Collier et al. for some MMP-collagen experimental systems using photobleaching. Measured diffusion coefficients are of the order of $4 \times 10^{-8} \text{ cm}^2/\text{s}$ and so movement along the 300 nm tropocollagen unit occurs on a timescale of tens of minutes to hours. Saffarian et al. computationally analyzed the ratcheting movement along the collagen fibril using a so-called random-walk ‘burnt-bridge’ computational model.

It is noted that extensive tendon damage leads to an increase in cell number and a change in phenotype, driven by various growth factors and their binding proteins including TGF-$\beta$, IGF-1, bFGF, PDGF, and others. As far as the authors are aware, there are no computational models of these signaling systems for tendon, although computational models have been developed in other tissues involving these signaling molecules. Note that only non-inflammatory repair processes are considered in the next section on repair.

**MODELING TENDON REPAIR**

In this section we constrain our discussion to non-inflammatory repair processes of Grades I and II ‘sub-failure’ damage, which we proposed is mediated by the confluent sheet of tenocytes surrounding a collagen fiber. It has been shown that the confluent sheet of tenocytes is stretched to the same degree as the collagen fibers are mechanically stretched. Stretching the collagen fibers and tenocytes stretches the tenocyte cytoskeleton, which in turn leads to changes in gene expression profiles of the tenocytes over time. This leads to a plausible local mechanism for close feedback control between mechanical stretching of the tissue and a tenocyte’s gene expression profile. Further as a corollary to this feedback process, it is not difficult to imagine localized damage would lead to a localized tenocyte stimulation and local fibril repair.

The localized non-inflammatory fibril repair is probably a self-assembly process driven by entropy minimization. In this process the tropocollagen produced by tenocytes self-assembles to form collagen sheets and fibrils. The collagen assembly process has been directly observed using the atomic force microscope. The process of self-assembly has been shown to be dependent on the local environment, including the temperature, redox potential, and pH of the primary fiber compartment. The self-assembly process within the primary fiber compartment is further coordinated by (autocrine or paracrine) growth factors, leading to the production of regulatory molecules like SLRPs. But there are many other regulatory molecules involved including fibronectin, integrins, perlecan, tenascin-X, thrombospondin, and various types of nonstructural collagens, as well as TIMPs. The nonstructural collagens V and XI are known to play a regulatory role by nucleating fibrillogenesis, while the SLRPs regulate fibril assembly and growth. There are five families of SRLPs that are homologous. Biglycan and decorin belong to the first SLRP family, and compete for the same binding site on collagen. Fibromodulin and lumican belong to the second SLRP family, and also compete for a binding site on collagen. These molecules help to regulate the distribution of fibril diameters and the cross-sectional shapes and fusion of fibrils within the collagen fiber. It is also noted in passing that SPARC expression patterns implicate this protein as an important mediator of collagen type I deposition and/or remodeling.

On the basis of our previous discussion, the critically important role of the confluent sheet of tenocytes that surrounds a primary fiber is now apparent. The confluent sheet of tenocytes coordinates and regulates all the fibrillogenesis and degradation processes within the collagen fiber compartment, thereby controlling fibril homeostasis. Computational models of these processes are likely to be critically important for understanding and then predicting the development of tendinopathies and predicting repair processes following surgery. As mentioned above, some computational models of the tropocollagen self-assembly process have been developed (see for example, Figure 7). At a fundamental level, the mer assembly process is regulated by controlling of the hydrophilic, hydrophobic and electrostatic forces between the binding molecules and the collagen. In principle, at a fundamental level, chemical free energy minimization, similar to the methodology developed by Israelowitz et al., provides a way to also model the regulatory mechanism for collagen fibril assembly.

Following tropocollagen self-assembly, covalent cross-linking between lysine residues and hydroxylysine residues (via aldehydes) binds the tropocollagen units together into the observable structural unit called a fibril. Importantly, the amount of cross-linking increases with age, so that young animals have relatively compliant tendons that become much stiffer and stronger with age. As far as the authors are aware,
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Having considered the repair of collagen fibrils, it is useful to consider how the degradation and repair processes (i.e., fibril homeostasis) may be coordinated by tenocytes over a diurnal cycle. Given the foregoing, it seems plausible to suggest that collagen is mechanically damaged by cyclic strain during the day. This is evidenced by the decrease in stiffness of the tendon observed between morning and evening. But the same mechanical process that damages the collagen fibril also protects the collagen fibrils from proteolytic degradation during the day. Further, it has been shown that mechanical stimulation of tenocytes through exercise (3 sets of 10 repetitions of 70% maximal leg extension) results in down-regulation of collagen type-I and MMP-2 production returning to normal after a 24-h period.

During the night when cyclic mechanical loading is absent, the damaged fibrils are repaired. To do this both tropocollagen production and protease expression by tenocytes is up-regulated. It is noted that recent experimental evidence for the eye has pointed to a prominent diurnal variation in the expression of metalloprotease/TIMP ratio (which increases during the night and decreases during the day), suggesting the closed eye is conducive to extracellular matrix remodeling. It is possible that similar changes take place in the tendon, perhaps for different reasons. Further, it is noted that tropocollagen produced by tenocytes may be broken down before it is incorporated into a fibril. During the night, MMPs bind where they can to collagen fibrils, and may bind preferentially to damaged collagen. The MMPs then move along the fibril and to find their preferred cleavage site, unwind the strands of the collagen triple helix, cut it, and release a fragment of the collagen mer to the surrounding extracellular matrix. The fragments are subsequently metabolized or excreted by the kidneys. Newly synthesized tropocollagen produced by the confluent sheet of tenocytes is incorporated into the fibril to effect a repair. This is evidenced by the increase in stiffness of the tendon observed between evening and the following morning. Finally exercise stimulates the coordinated synthesis of collagen in both muscle and tendon. As far as the authors are aware, no computational model of degradation and subsequent repair based on the known and interpolated physiological principles has been developed to date.

CONCLUSION

There have been many computation models developed that are relevant to tendon. Some of the most notable
computational modeling achievements to date have been:

1. tropocollagen formation into a triple helix driven by minimization of chemical potential energy;\(^34\);
2. molecular dynamics model of tropocollagen load-deformation response;\(^35\);
3. discrete models of tropocollagen aggregation and the load-deformation properties of the aggregates;\(^36,37\);
4. discrete model of collagenase binding to a collagen fibril and the diffusing along it;\(^91\);
5. continuum model of collagen fibril degradation by proteases;\(^86\);
6. various composite solid,\(^1,63,64\) viscoelastic,\(^65–67\) and poroelastic\(^46,47,69\) continuum models represented the time-dependent load-deformation characteristics of tendon;
7. various hyper-elastic models\(^63,64,69–71\) representing the large deformation response of tendon, sometimes spanning a couple of length scales;
8. hyper-elastic damage models\(^69,70\) of tendon, that can capture strain softening;
9. hyper-elastic damage and repair model\(^70\) of tendon, capturing some aspects of structural changes within the tendon;
10. probabilistic mechanical models that include the distribution of fiber lengths, and probabilistic damage models capturing extension-damage behaviors.\(^69,74\)

However, owing to limited conceptual understanding of how the tendon tissue actually functions and an absence of a deep understanding of the processes leading to tendon homeostasis, there are many significant gaps in model development. In the above, we have emphasized the importance of compartments in tendon, the blood supply of tendon, and the repair processes in tendon. To date there is a need to develop models of:

1. realistic mechanical damage models of tendon that take into account the initial state of the tendon, and loading magnitude, frequency and pattern of loading;
2. interactions between strain and tenocyte responses including synthesis, proliferation, and differentiation;
3. realistic models of fibril assembly and regulation;
4. regulation of fibril repair and proteolytic degradation by tenocytes;
5. interaction between strain and proteolytic degradation;
6. interactions between blood supply and mechanical deformation;
7. the transport of molecules from blood vessels to the site of tissue action;
8. cyclic damage and repair processes represented on realistic timescales in a diurnal pattern;
9. realistic multiscale predictive models based on the structure and compartments of Achilles tendon.

We believe that developing any of the above models would help address important questions in tendon homeostasis. We propose that if the question to be answered demands the development of a general multiscale tendon homeostasis model (e.g., how does tendinosis of the Achilles tendon development), then ideally it should incorporate all these sub-models. Such an integrated model will lead to new insights into the processes of tendon homeostasis, particularly how processes interact across length and timescales. This would likely direct further experiments, aiding the process of hypothesis development, but as importantly it would provide a valuable tool to probe important components of the process that may determine tendon health and disease.

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