Tendon explant models for physiologically relevant in vitro study of tissue biology – a perspective

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ABSTRACT

Background: Tendon disorders increasingly afflict our aging society but we lack the scientific understanding to clinically address them. Clinically relevant models of tendon disease are urgently needed as established small animal models of tendinopathy fail to capture essential aspects of the disease. Two-dimensional and three-dimensional cell and tissue culture models are similarly limited, lacking many physiological extracellular matrix cues required to maintain tissue homeostasis or guide matrix remodeling. These cues reflect the biochemical and biomechanical status of the tissue, and encode information regarding the mechanical and metabolic competence of the tissue. Tendon explants overcome some of these limitations and have thus emerged as a valuable tool for the discovery and study of mechanisms associated with tendon homeostasis and pathophysiology. Tendon explants retain native cell-cell and cell-matrix connections, while allowing highly reproducible experimental control over extrinsic factors like mechanical loading and nutritional availability. In this sense tendon explant models can deliver insights that are otherwise impossible to obtain from in vivo animal or in vitro cell culture models. Purpose: In this review, we aimed to provide an overview of tissue explant models used in tendon research, with a specific focus on the value of explant culture systems for the controlled study of the tendon core tissue. We discuss their advantages, limitations and potential future utility. We include suggestions and technical recommendations for the successful use of tendon explant cultures and conclude with an outlook on how explant models may be leveraged with state-of-the-art biotechnologies to propel our understanding of tendon physiology and pathology.

1. Introduction

1.1. Clinical need and role of tendon explant models

Musculoskeletal diseases accounts for over 1/3 of human years lived with disability, with tendon disorders representing 30–50% of all musculoskeletal-related clinical visits. Tendon disorders are associated with pain, swelling and restricted ranges of motion, are usually exacerbated by activity, and affect individuals across the demographic spectrum. The most common tendon disorders involve the Achilles, patellar, rotator cuff, digital flexor, and elbow extensor tendons, often leading to despair for patients and frustration for the clinicians trying to treat them. Unfortunately, the societal prevalence of tendon disorders is only increasing, in line with major risk factors including age, obesity, diabetes, mechanically demanding work and sport activities. Yet despite ever-growing rates of incidence, substantial individual suffering, and an enormous collective socioeconomic burden, tendon disorders remain under-researched and poorly understood.

Current clinical approaches to treating tendon ailments involve pain management and physiotherapy over a course of several months, sometimes extending into years. The ability to introduce more effective therapies against tendon disease has been slow due to lacking knowledge on the molecular and cellular basis of tendon pathology and physiology.

A major obstacle to scientific and clinical advancement has been the persistent lack of valid models of human tendon disease. Such models are essential to clarify basic cellular mechanisms and to test novel therapeutic treatments. In this review, we discuss the potential of tendon explant models to fill this gap. They are uniquely powerful because they (1) preserve the cell’s native extracellular matrix (ECM) niche, (2) they can be viably maintained and longitudinally monitored for extended periods of time in ex vivo culture, (3) their physiology can be probed using available tools from the fields of cellular and molecular biology, and critically (4) the mechanics and structure of the (dys)functional matrix can be quantitatively phenotyped in a multi-scale fashion, providing a clear...
functional context in which to interpret and explain observed cellular behaviors.

1.2. Structure and function of the tendon extracellular matrix niche

While the cellular underpinnings of tendon physiology still remain mostly obscure, the manner by which the tendon ECM delivers its mechanical function is well understood. Tendon serves to transfer muscle force to bone, enabling movement and/or musculoskeletal stability. Healthy tendon tissue features a hierarchically structured collagen matrix that is anatomically optimized to sustain extraordinary mechanical demands. The tendon ECM houses tendon cells within a complex niche that can be very roughly divided into an electron-dense load-bearing collagen network embedded within a mostly non-collagen matrix (Figure 1(a)).

The dense tendon ECM comprises tightly packed fibrillar collagen molecules (thought to be dominantly type-I collagen amongst a potentially wide range of other collagen types) packed into so-called collagen fibrils with diameters ranging from 10s to 100s of nanometers and with lengths reaching to the centimeter scale\textsuperscript{17,18}. The fibrils are typically bundled along their load-bearing axis into cellular size scale structures called collagen fibers that are visible under a light microscope. These fiber bundles, and the cells that maintain them, in turn, constitute the tendon fascicle—considered to be the basic functional unit of tendon tissue. The tendon fascicle is also the smallest unit of tendon tissue useable as a tissue explant\textsuperscript{17}.

Aside from type I collagen structures, the pericellular matrix surrounding a tendon stromal fibroblast

![Figure 1](image_url)

**Figure 1.** The tendon cell’s extracellular niche at a glance. (a) Tendon tissue comprises aligned collagen-rich fascicles interspersed with tendon stromal cells that reside in a highly complex/structured ECM niche. The interstitial matrix is mainly formed by collagen type I fibers, which constitute the most abundant ECM component in tendon and embrace the cells longitudinally. The non-collagenous matrix is rich in glycoproteins, in tendons mainly represented by the small leucin-rich proteoglycans (SLRPs). The pericellular matrix is a thin, continuous layer surrounding the cells and that contains collagen types V and VI, elastin and fibril associated collagens with interrupted triple helices (FACITs); (b) In healthy adult tendon tissue, the cells are closely connected to one another and remain in a quiescent state. In diseased tendon tissue, cell and ECM homeostasis are disturbed leading to cell activation and abnormal ECM production and turnover.
comprises a complex but poorly understood milieu. The fibrillar collagen types III, V and XI are typically only present in small quantities in healthy tendon. While the full biological and biophysical role of many of these collagens is still unclear, collagen type III fraction within the ECM increases during tendon healing, and is considered to serve as a provisional matrix. Collagens types V and XI are known to regulate collagen fibril formation in the processes known as fibrillogenesis. Several non-fibrillar collagens are also present in tendon in low amounts, for instance, type VI is often localized to the pericellular matrix and is increased in abundance in injured tendons. The fibril-associated collagens (FACITs) types XII and XIV are described as providing a molecular bridge between type I collagen and other matrix molecules, and play important roles during tendon development.

The non-collagen components of the matrix intersperses the collagen-dense structures at each hierarchical level. This hydrated interfibrillar matrix spans single collagen fibrils, fibers, and fascicles, encouraging fluid flow and nutrient transport, as well as accommodating interspersion of nerves and vessels. These wrapping layers are rich in proteoglycans (a distinct subgroup of glycoproteins), in tendon mainly represented by the small leucine-rich proteoglycan (SLRP, e.g. decorin, biglycan, fibromodulin, and lumican), versican and aggrecan, and contain a small fraction of elastin and other glycoproteins including fibronectin, laminins, lubricin, tenasin C and members of the thrombospondin family (e.g. cartilage oligomeric protein, also referred to as thrombospondin-5). This diversity of structural matrix proteins broadens the functional range of the tissue beyond tensile load-bearing, contributing to fiber and fascicle sliding, providing resistance to compression, supporting collagen matrix assembly and regulating cellular processes during tendon development and healing. The quantitative and spatial distribution of matrix constituents varies between different tendon regions (myotendinous junction—mid-substance—enthesis) and tendon types (load-bearing and positional), with the roles of the various matrix component being an active area of research.

The tendon ECM is synthesized, maintained and remodelled by a still poorly defined population of fibroblast-like stromal cells. Tendon stromal cells are traditionally characterized by an expression of mechanically regulated transcription factors such as scleraxis and mohawk that are essential to normal tendon development, as well as the expression of the glycoprotein tenomodulin. While scleraxis gene expression is perhaps most widely used as a tendon cell marker, it has a largely unknown function and is expressed in several tissues, such as the skin, heart and the cornea. Recent studies, however, show that the tendon stroma comprises a heterogeneous cell population, including resident fibroblasts, macrophages, and stem/progenitor cells, where a clear picture on the individual cellular phenotypes, their origin and function is still emerging and urgently needed.

Tendon stromal cells are found interspersed between collagen fibers where they align longitudinally allowing effective cell-cell and cell-ECM communication. Together with their local collagen fiber network, tendon stromal cells form the fascicles defined as the load-bearing tendon core.

### 1.3. The extracellular matrix niche in tendon health and pathology

Considering the largely mechanical function of tendon and the extreme mechanical demands on tendon tissues, cellular sensing of mechanical signals is an essential aspect of tissue homeostasis. Mechanical loads are transmitted to tendon cells via their local matrix, with these cues guiding cell interactions with the matrix in a feedback loop. Within the cell, mechanically regulated gene expression patterns and cell metabolism orchestrate protein synthesis (or post-translational modification) including ECM proteins, growth factors, cytokines, and proteases. This process shapes the cellular microenvironment, tuning cell-cell and cell-matrix interactions with an impact on homeostatic balance. When these processes go awry, tendon tissue may shift (in whole, or in part) from a healthy to a dysfunctional state. Indeed, histopathological studies have clearly shown that tendon disease states are characterized by cell activation and substantial changes in ECM components and organization (Figure 1(b)). Alterations include hypercellularity, neovascularization (presence of a fibrovascular ECM), inflammation and a dysregulation of the critical balance between ECM remodeling proteases (MMPs, ADAMTs, cathepsins) and their inhibitors. When sustained these changes most probably reflect failed cellular efforts to re-establish tissue homeostasis, including an adequate restructuring of the tendon matrix with sustainably embedded resident cells. Hence, tendon tissue behavior is tightly dependent on specialized biophysical and biochemical niches defined by the ECM architecture, ECM biophysics, and context-dependent cell-matrix interactions. These aspects of the extracellular niche are essential to consider in any research model that is aimed at studying tendon homeostasis and mechanisms of pathology.
It is extremely difficult, if not impossible, to adequately recapitulate original ECM characteristics in vitro using engineered two-dimensional (2D) or three-dimensional (3D) culture systems. Very importantly, tendon explant models include these key features of the native tendon microenvironment. As such they are exceptionally well-suited for in vitro study of cell-cell and cell-matrix signaling—allowing insight into cellular communication that cannot be reasonably studied on cells in a simulated ECM niche. In the following sections, we make the case for increased use of explant models to uncover basic mechanisms of tendon physiology.

2. The utility and power of tendon explant culture models

2.1. Limitations of tendon in vivo and in vitro models

Studies on basic mechanisms of tissue physiology largely derive from either animal models or from in vitro experiments using isolated tendon cells. Although animal models are commonly viewed as a gold standard for the study of tendon repair, these models are limited in their ability to capture features of human tendon disease, for instance, they fail to mimic the limited intrinsic repair capacity of adult human tendon tissue. Tendon tissue response to an acute injury or mechanical overuse is species-specific, involving systemic healing processes with an often-unclear homology to humans. This seems to be particularly problematic in small animal models of tissue repair that can deviate from human system behaviors in centrally important ways, for instance in immune system involvement in healing. On the other hand, large animal models, such as horses, dogs and sheep, naturally develop tendon disease, thus better resembling the human condition. Moreover, the large size of these animals facilitates the implementation of initiation defects and the mimicking of human tissue response to an implanted biomaterial. Yet, high housing and animal care costs, long breeding times and late skeletal maturity translate to low throughput and limit the practical use of large animals in tendon research.

Apart from the above-mentioned limitations, the use of animal models also introduces considerable ethical and time constraints. And although in vivo models are attractive for investigating tissue development and tissue repair, complex systemic regulation of tendon physiology and pathology presents many unknown yet potentially decisive confounding factors that cannot easily be controlled.

At the other end of the spectrum, 2D and 3D cell culture systems are substantially less complex than in vivo models and are widely used due to their practicality. Cell-based models are powerful and attractive in the sense that they accommodate the use of human cells, potentially allowing investigators to capture clinically and biologically relevant effects of human individuality (donor-to-donor variability). However, cell culture models come with many limitations. One major drawback is the loss of proportional heterogeneity from the isolated cell population during in vitro cell expansion. Such imbalanced conditions hamper drawing conclusions on whole tendon biology if only individual, more adhesive/proliferative cell subpopulations are selected in vitro. How to prevent or compensate for this loss is an active area of research, particularly how culture conditions influence the selection and growth of individual cell phenotypes. Nevertheless, cell-based models are particularly useful for high-throughput screening of response to experimental conditions and are in fact well suited for studies of robust cellular mechanisms that are relatively insensitive to physiological context. The physiological contexts that in vitro 3D models can adequately capture arguably includes neo-tendon formation after an acute injury, the study of initial interactions between recruited tendon cells and an implanted biomaterial or the crosstalk between tendon fibroblasts and other cell types (e.g. immune and stem cells). On one hand, cell models have been widely used to study cellular sensitivity to biochemical and biophysical cues from the cellular niche (model ECM ligands, mechanical substrate compliance, drugs). On the other hand, speculating on the biological relevance of the behaviors that emerge within cell models presents risks that often requires verification using other model systems and validation against clinical evidence.

2.2. Advantages of tendon explant models

When used appropriately, ex vivo explant models enable insights that cannot be gained using other in vitro and in vivo models of tendon physiology (Figure 2). Whole tendon tissues maintained in viable culture conditions comprise original cell subpopulations with physiological cell organization, native ECM composition and native ECM architecture. In tendon explants, cells reside within a physiological ECM niche that can transfer mechanical stimuli and biological signals in a manner that largely mimics the complexity of cell-cell and cell-matrix communication. This allows highly controlled yet biologically relevant studies on cell-driven ECM turnover processes within the tissue stromal compartment. It also opens the possibility to directly investigate tissue functionality by measuring mechanical properties and initial aspects of crosstalk between tendon stromal tissues and other
tissue compartments that encompass the immune, vascular, and nervous systems.

In comparison to in vivo models, explant models permit the deep study of the biological response of stromal tissues to external factors in a manner that the factors can be better isolated and controlled. In models that provide an abundance of explantable tissues (e.g. rodent tail tendon explant models), a range of experimental conditions can be investigated with minimal pain and distress to the animal. In being both scalable and highly reproducible, tissue explant models adhere to the advancement of the “3Rs” principle for animal experimentation (Replace, Reduce, Refine)\(^66\). In this sense, explant model systems can mitigate some ethical limitations of in vivo models, while simultaneously alleviating economic and time-related burdens.

Another major technical advantage of explant models is that molecular tools and techniques that have been developed for standard cell culture experiments can be readily applied. This allows deep probing of molecular pathways, as well as high-throughput functional screening on both the tissue and molecular levels—a highly powerful combination. First, of course, the physiological relevance of the explant culture system must be established by convincing agreement with clinical experience and insights derived from the study of human tissue. After relevance is established there is immense potential to exploit these models for the identification of novel mechanisms that may translate to clinical strategies for disease prevention and/or the corresponding development and testing of new therapeutics.

### 2.3. Scientific advancements enabled by tendon explant models

#### 2.3.1. Systems to study tissue crosstalk

Tendon explants have long been productively used for the \(\textit{ex vivo}\) characterization of collagen tissue structure–function relationships attributed to the ECM\(^67\text{--}76\).
Concomitant with ever-improving understanding of tendon cell homeostasis and our ability to physiologically maintain it in vitro, tendon explant models have been increasingly used to study cell-mediated processes. These processes necessarily include crosstalk between cells, their ECM and their nutritional environment, aspects that all closely interact to regulate tissue turnover in health, disease, and healing. For instance, explanted canine flexor tendons have been harnessed to verify clinical impressions that retention of the tendon sheath is beneficial to healing after surgical repair of lacerated tendons. This work demonstrates both the clinical value of explant models, and highlights the possibility to employ them for the discovery of basic mechanisms. Other examples demonstrating the potential of explant models include indirect co-culture approaches using human adipose-derived stem cells and human tendon explants that point to bi-directional crosstalk with tendon models demonstrate their value in isolating tissue- and cell-specific contributions to tendon physiology.

Perhaps the real power of explant model systems is that they enable investigation of tissue crosstalk. Tendon explants can be exposed to soluble factors to reveal cellular response within their native 3D matrix structure. As a recent example, a rat rotator cuff injury model for investigating bone-muscle-tendon crosstalk was introduced to investigate the role of inflammatory mediators on cell viability and ECM structure. Our own group has exploited equine tendon explants to investigate cellular sensitivity to multiscale changes in tendon matrix mechanics induced by exogenously applied cross-linking agents. Others have investigated the effect of growth factors and/or hormones (e.g. platelet-derived growth factor, dexamethasone) on the metabolism of cells within their 3D matrix using rabbit flexor tendons, equine superficial digital flexor tendons and human explants. While most studies to date have focused on fairly narrow functional readouts of cells (e.g. viability, gene expression) or their matrix (e.g. elastic modulus), explant models offer a large untapped potential for deeper biological investigation. We believe that *ex vivo* systems represent a powerful approach for the study of multiscale tissue properties and the characteristics of cells in their native 3D environment.

### 2.3.2. Mechno-culture systems

Given the mechano-sensitive nature of tendon in general and the fact that mechanical overuse is considered to be a driving factor in many tendon disorders, the inclusion of (cyclic) mechanical stresses is likely to be an essential requirement for faithful replication of the *in vivo* tendon niche. Appropriate experimental strategies are needed for this endeavor, as studying (healthy) human tendon tissue *ex vivo* is generally impossible. Mechanical stimulation of tendon models via controlled mechanical stretching systems are well-suited methods to simulate the effects of physiological or pathological loading on cells and ECM. Mechanical stretching systems may also be coupled to environmental conditions to mimic specific *in vivo* cellular and tissue niches in a standardized and controlled fashion. While mechanoculture systems for cell culture are widely used and well established, tissue-specific mechanoculture of 3D tissues is more challenging. In the tendon field, both commercially available and custom-designed bioreactor systems have been implemented over the past three decades. Our laboratory is among those pursuing this work, having recently described a tendon specific bioreactor system for long-term mechano-culture that allows individual specimen culture conditions and including tissue pre-tension. We see a great potential in exploring explant mechano-culture within specific biochemical niches as important to establish a baseline for physiologically relevant studies on tendon homeostasis, disease, and recovery.

However, a major challenge of tendon explant research is that many details regarding how different loading conditions (magnitude, frequency, rest) affect tendon development, homeostasis, damage, and repair remain unknown. Disparities in models, methods, and reporting of results have added fuel to the debate on exactly what constitutes physiological mechanical stimulus, and what constitutes pathological mechanical overload. Although it is widely accepted that chronic tendon problems arise from the excessive tensile load, excessive compressive forces (such as due to bony impingement) have been proposed as one specific cause of tendon disorders. Further, tissue shear strains can yield various combinations of tensile and compressive loading at the cellular level, and may result in aberrant stimulus or even damage at the cellular level. Yet, past research has generally attributed tendon pathology to tensile overload because isolating a cell-level mechanical stimulus is difficult and so remains a major challenge in the field. However, explant models used in combination with functional microscopy are already revealing the relationships between tissue-level mechanical stresses, cell-level deformations, and central cellular mechanisms of mechanotransduction.

Despite opaque underlying cellular mechanisms, explant models have been productively employed to explore the strong link between tissues loads and biological response. For instance, experiments using avian flexor...
digitofus tendon have indicated that aggressive mechanical loading results in a drastic reduction of tendon mechanical properties that are associated with higher secretion of collagenase and inflammatory mediators, such as prostaglandin E2 and nitric oxide$^{103,104}$. Likewise, supra-physiological loading introduced areas of ECM damage in equine superficial digital flexor tendon and an increase of factors associated with matrix turnover, such as matrix metalloproteinase 13 (MMP-13) and C1,2C$^{105}$. Additionally, cyclooxygenase-2 (COX-2) and interleukin-6 (IL-6), both inflammatory markers, have been reported to be elevated in overloaded tendon ex vivo. Up-regulation of inflammatory (IL-6) and matrix degradation markers (MMP-3, MMP-13, C1,2C) in explants of bovine deep digital flexor tendons has confirmed these findings$^{106}$. Similarly, by correlating ECM properties to such molecular markers, it was shown that increased IL-6 gene expression in explanted bovine extensor tendon fascicles occurs after application of a minimal damage loading protocol, indicating that this behavior is robustly modeled in explants even across species$^{107}$. Remarkably, the increase of mediators associated with inflammation and matrix remodeling is supported by observations of pathological human-derived tissues, implying that certain proinflammatory features of tendon disease are captured by ex vivo tendon overloading$^{108,109}$.

Complementing explant studies of supra-physiological loading, many studies have investigated sub-physiological loading. Removal of a mechanical stimulus upon explantation has been reported to dramatically increase MMP expression and secretion followed by a decrease of biomechanical properties in explanted canine, rabbit and rat tendons$^{86,110}$. However, the response to changes in mechanical loading may be tendon-type specific as shown by comparing mechanically modulated gene expression in Achilles and supraspinatus tendon of rats$^{112}$. Our own studies using mouse tail tendon explants have revealed that this mechanically gated proteolytic response to unloading is strongly dependent on the culture conditions$^{89,118}$, a context-dependency we speculate may be related to relative tissue vascularity. We believe these studies highlight how explant culture systems can be productively and powerfully utilized for investigation of tendon mechanobiology, allowing controlled application (or deprivation) of mechanical loads and the longitudinal functional tracking of mechanical properties.

### 2.4. Tendon fascicles—a unique tool to study the tendon stromal core

Within a given tendon, fascicles represent “base functional units” that are hierarchically ordered into core structures in a manner that provides a wide potential range of tissue level biomechanical properties$^{77}$. For the study of these functional units, rodent tail tendon fascicles are ideally suited. While few groups have mastered the art of extracting and exploiting highly cross-linked load-bearing tendon fascicles$^{106,114}$, tail tendon fascicles can be readily extracted with minimal mechanical or biological damage. Rat and mouse tail tendons have thus played an important role in studies of tendon structure–function relationships (e.g.$^{100,115,116}$), mostly because of their ease of isolation, a high yield of fascicles per animal, and a high degree of mechanical and biological reproducibility$^{117}$. Furthermore, rodent tail tendon explants represent a powerful model to study mechanically regulated matrix remodeling$^{89,118}$. The tail fascicle has well-aligned collagen structures with clearly defined tissue boundaries. This structure minimizes the chances of preparation artifacts and is favorable for the application of controlled loading regimes (tensile over- or underloading) to examine the effects of mechanobiological stimuli$^{111,119}$. It is, however, important to note that cells within explanted fascicles can be damaged by supra-physiological but subrupture mechanical loads, and that micro-damage to the extracellular matrix of rat tail tendon fascicles is not recovered after several days of in vitro culture$^{120}$. We speculate that the limited healing capacity of isolated tendon fascicles may be due to a lack of progenitor cells that are present within the interfascicular matrix. We view this as a major advantage that makes the fascicle an ideal model to isolate and investigate tendon core response to extrinsic influences. Straightforward stress-deprivation and/or static tension of tail tendon fascicles have already provided valuable understanding of mechanisms that are implicated in the development of pathology, healing and tissue homeostasis$^{50,94,111,118,119,121–125}$. We thus view rodent tail tendon explants as representing the ideal combination of experimental reproducibility and cross-species relevance for use as an in vitro model of “core tendon” biology.

Nonetheless, potentially important concerns have been raised regarding the physiological and clinical relevance of rodent tail tendons as a model for human disease and repair$^{77,117,126}$. The most cited shortcoming is the fact that human pathologies typically occur in load-bearing tendons while rodent tail tendons are positional tendons. Load-bearing and positional tendons have functionally related differences at both the cellular and compositional levels, for instance, major differences in the nature and extent of collagen cross-linking. However, the relevance of these differences is debatable, as highlighted by studies demonstrating that load-transfer and damage mechanisms are comparable in load-bearing and positional tendons$^{99}$. Among other important similarities, the tail fascicle has mechanical properties that are comparable to
mature load-bearing human tendon, with elastic moduli ranging from several hundred MPa to over 1 GPa72,117,128. Additionally, the failure properties of isolated tail tendon fascicles reflect those of whole human tendon, with failure stresses on the order of 80 MPa and failure strains of approximately 10%72,128. Aside from the molecular differences in collagen cross-links (divalent aldimine cross-links in murine tail tendon tissue versus combined divalent aldimine and trivalent histidine cross-links in human), the ECM biochemical composition and architectural structure of rodent tendon is similar to healthy human tendon50,73. Similarly, the genetic regulation and protein composition involved in native tissues and in response to a tendon injury in certain mouse strains is remarkably close to that of human tendon—suggesting that many of the basic biological mechanisms of the tendon are conserved across species and anatomical origin129. In view of available evidence, we conclude that the tail tendon model is well suited for investigations within non-damaging physiological ranges of mechanical loading.

2.5. The challenges of maintaining tendon homeostasis in explants

Despite its many advantages, work with tendon explants comes with challenges. A central challenge is to understand the conditions required for tissue homeostasis. Very little is known about the healthy tendon environment, including mechanical, biological and physico-chemical factors that should be included to or excluded from culture conditions. Importantly, the very definition of tissue homeostasis remains a crucial topic. Because healthy adult tendon tissue is metabolically calm130–132 and becomes activated in disease, we believe that culture conditions should enable a homeostatic baseline in which tendon cells remain quiescent. To potentially achieve tendon homeostasis ex vivo, we believe the community must first uncover the full range of cellular and molecular changes that occur in tendon upon explantation. This major effort requires unbiased screening experiments on genes, proteins, metabolites and kinase activation with respect to the ex vivo culture conditions. If quiescence is unreachable, the anabolic and catabolic processes should at least be in balance as to maintain a steady-state of mechanical (functional) tissue properties over time. Indeed, such homeostatic-like conditions can be achieved by loading tendon tissue89,110, paying special attention to choosing experimental culture conditions (media composition, culture gas composition, and the physical stimulation environment; see below) or by inhibiting catabolic processes125,133.

Our own efforts to coax tendon explants toward functional recovery from imposed tissue damage have been challenging120. This difficulty is implicitly confirmed by studies in the literature that almost exclusively rely on proxy measures of anabolic tissue response (i.e. cellular gene and protein expression) but which do not demonstrate the formation of functional matrix (for example58,134–136). We nonetheless hold hope that a functional repair response can be achieved in tendon explant models, as has been achieved in other explanted model systems including spinal cord, meniscus and cochlear tissues137–139.

3. A perspective on the utility and power of tail tendon explants as a model system for investigating tendon biology

The shift from homeostatic cell behavior after explant extraction from the host is poorly understood. However, it is well known that gene expression dramatically changes immediately after extraction and that the process of matrix proteolysis begins. From our own experience, we have learned that factors such as tissue temperature and oxygen tension strongly determine the outcome of explant cultures113. By finding an appropriate loading regime and culture conditions that mimic in vivo conditions we may be able to limit such molecular shifts and approach the critical task of better understanding tendon homeostasis84. Along these lines, it may then also be possible to extend the currently limited viability of ex vivo culture models. Monitoring cell viability in explant culture is important, as the release of inflammatory cytokines from apoptotic cells may trigger cell death in adjacent cells, which finally distort the experimental outcomes81,140. Additionally, it is essential to examine whether it is valid to extrapolate results from explant model systems to draw conclusions on human tendon biology and disease.

While existing long-term explant culture studies on tail tendon mostly employ rat tissue, our work on murine tail tendon explants is the first to explore this model84,113. This is likely because mouse tendons are relatively small and fragile, which poses challenges in their handling. However, murine tail tendons offer several advantages: (1) their small size allows consistent tissue gradients of oxygen and nutrients from the margins to the center of the explant, (2) working with murine tissue is highly cost efficient and perhaps most importantly, (3) mouse models offer obvious advantages in terms of the available genetic models and molecular tools.

In this regard, our work with murine tail tendon fascicles has demonstrated their utility for controlled
parametric investigation of tendon physiology and pathology. These initial efforts have focused on the maintenance of tissue homeostasis. In absence of tension and in standard cell culture conditions (10% serum, 37°C, 20 kPa \( \text{pO}_2 \)), mechanical tissue degradation in unloaded murine tail tendon fascicles is readily detectable after 1 week of culture. These results are consistent with earlier studies showing that mechanical unloading leads to functional tissue breakdown, a process that can be abrogated by application of mechanical tension. While the number of parameters that may be varied in the culture environment to maintain tissue stability are numerous and depend on the research question, our investigations have shown that various combination of tissue loading, oxygen tension, temperature and presence of serum can alternately promote or inhibit matrix breakdown.

3.1. Mechanical loading

It is difficult to draw conclusions regarding best practices for mechanoculture intended to maintain tendon explant homeostasis. An ideal protocol will likely depend on the anatomical role of the tendon, among other factors. However, for positional tendons, such as murine tail tendon, it appears that loads in the range of the toe region in the stress–strain curve (e.g. those that yield crimp disappearance) are sufficient to maintain tissue mechanics at levels close to native tendon fascicles, and without inducing mechanical damage to the cells or matrix. Arnoczky and colleagues discovered that the proteolytic erosion of mechanical properties can be delayed by simply anchoring the tendon ends to promote tensile homeostasis. While it seems that static anchoring can maintain functional properties of the tissue, the protective effects of tissue tension are not only cellular, as tissue tension can obscure enzymatic cleavage sites and can protect the collagen matrix from breakdown.

3.2. Oxygen partial pressure

Our most recent work has focused on the role of tissue vascularity in driving cells from homeostasis and activating them toward matrix turnover. Healthy tendon cells reside in a niche with physiologically low oxygen availability. However, standard cell and tissue culture is normally performed at atmospheric oxygen partial pressure, which considerably exceeds the physiological condition that cells normally encounter in vivo. On this basis, we suggest that maintaining tendon explants at oxygen partial pressures around 3 kPa better approximates a healthy tissue environment.

3.3. Temperature

We have observed that culture temperature significantly influences explant stability, and we view it as imperative that one considers the relative physiological temperature of the tissue origin (core or extremity) when choosing the appropriate culture temperature for maintaining explant homeostasis. While the culture temperature will affect biological processes ranging from enzymatic activity to mass transport, it can also affect the biophysical stability of the extracellular matrix. Denaturation temperature and mechanical properties of different tendons significantly depend on the number of cross-links present in a tissue. The type of cross-links only indirectly affects the denaturation temperature of a tendon, namely through their effect on the hydration of the tissue (with a negative correlation between hydration and stability). The fact that cross-links vary between tendons of different origins and develop with maturation may reflect the local thermal environment of maturation. The local thermal environment of the tissue, again highlighting that physiological explant culture conditions will vary depending on the source of the tendon.

3.4. Serum

Serum contains a wide range of nutrients, hormones and growth factors. It is a poorly defined culture medium component containing ingredients that may or may not be physiologically relevant for maintaining tendon homeostasis. Because the healthy tendon is generally nutrient poor due to limited blood flow within the tendon core, we believe that a serum-free culture conditions mimic a healthy tendon microenvironment more suitably than serum-supplemented conditions. We have recently shown that serum may contribute to tissue degradation depending on the other above-mentioned culture parameters. Additionally, serum promotes cell proliferation and migration out of the tissue, a hallmark generally encountered in pathological tendon tissue.

4. Conclusions and perspective

Tendon explant models are a potentially powerful tool for research on human tendon health, damage and repair (Figure 3). Unlike all other in vitro model systems, explants retain important features of healthy tendon tissue including cell-cell and cell-matrix connections that are central to tissue regulation and
essential for tissue homeostasis. Tendon explants incorporate a near-native microenvironment for tendon cells while allowing a reduction of the systemic complexity that generally clouds interpretation of in vivo model responses. Finally, the ability to track biological readouts (such as live imaging) in parallel to direct measurement of functional (mechanical) tissue properties represents a major advantage of explant models. We view tendon fascicle explant models to offer an ideal foundation for investigating tissue crosstalk. Here the fascicle can be modularly combined with cell models and/or other tissue models, gradually and controllably increasing model complexity. For instance, co-culture models of tendon explants with endothelial or immune cells eventually embedded in supporting hydrogel matrices can be used to study compartment crosstalk between the tendon core and its extrinsic environment.

Further, gene-editing methods (e.g. CRISPR/Cas9) allow within targeted studies to focus on the role of altered cell-cell and cell-matrix communication in tendon damage and repair. We envision that, over time, successful model systems will emerge that are able to capture clinically relevant features of tendon health and disease. We believe that such models will find increasing use within pharmacological high-content screening approaches. Ultimately, we expect that tendon explant models will play an integral role in unraveling biological mechanisms of tendon disease and accelerate the clinical translation of new therapies.

Disclosure statement
No potential conflict of interest was reported by the authors.
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