TOPICAL REVIEW

Tendon tissue engineering: biomechanical considerations

To cite this article: Devina Jaiswal *et al* 2020 *Biomed. Mater.* **15** 052001

IOP Publishing Biomed. Mater. 15 (2020) 052001 https://doi.org/10.1088/1748-605X/ab852f

Biomedical Materials

TOPICAL REVIEW

Tendon tissue engineering: biomechanical considerations

Devina Jaiswal[1], Laurie Yousman[4], Maxwell Neary[2], Emily Fernschild[1], Brennen Zolnoski[1], Sara Katebifar[2], Swetha Rudraiah[3], Augustus D Mazzocca[4] and Sangamesh G Kumbar[2,4]

[1] Department of Biomedical Engineering, Western New England University, Springfield, MA, United States of America
[2] Department of Biomedical Engineering, University of Connecticut, Storrs, CT, United States of America
[3] Department of Pharmaceutical Sciences, University of Saint Joseph, Hartford, CT, United States of America
[4] Department of Orthopedic Surgery, University of Connecticut Health Center, Farmington, CT, United States of America

E-mail: devina.jaiswal@wne.edu **and** kumbar@uchc.edu

RECEIVED
15 October 2019

REVISED
28 March 2020

ACCEPTED FOR PUBLICATION
31 March 2020

PUBLISHED
15 July 2020

Keywords: tendon, ligament, bioreactors, mechanosensory, mechanical-stimulation, tissue engineering, regenerative medicine

Abstract

Engineered soft tissue products—both tendon and ligament—have gained tremendous interest in regenerative medicine as alternatives to autograft and allograft treatments due to their potential to overcome limitations such as pain and donor site morbidity. Tendon engineered grafts have focused on the replication of native tendon tissue composition and architecture in the form of scaffolds using synthetic or natural biomaterials seeded with cells and factors. However, these approaches suffer due to static culture environments that fail to mimic the dynamic tissue environment and mechanical forces required to promote tenogenic differentiation of cultured cells. Mechanical stimulation is sensed by cellular mechanosensors such as integrins, focal adhesion kinase, and other transmembrane receptors which promote tenogenic gene expression and synthesis of tendon extracellular matrix components such as Type I collagen. Thus, it is imperative to apply biological and biomechanical aspects to engineer tendon. This review highlights the origin of tendon tissue, its ability to sense forces from its microenvironment, and the biological machinery that helps in mechanosensation. Additionally, this review focuses on use of bioreactors that aid in understanding cell-microenvironment interactions and enable the design of mechanically competent tendon tissue. We categorize these bioreactors based on functional features, sample size/type, and loading regimes and discuss their application in tendon research. The objective of this article is to provide a perspective on biomechanical considerations in the development of functional tendon tissue.

1. Introduction

Tendon connects bone and muscle, facilitating joint movement by transferring forces. Tendons such as the flexor, rotator cuff, or Achilles commonly suffer ruptures due to high impact events (such as high stress athletics or laceration due to a sharp object) or tendinosis due to age-related tissue degradation or overuse. According to the National Institutes of Health (NIH), out of 33 million yearly musculoskeletal injuries, 50% are tendon or ligament injuries (Wu *et al* 2017a). Similar to other tissues, tendon undergoes a wound healing cascade, however, the self-regeneration process suffers due to lack of blood vessels and low cellular content (Thomopoulos *et al* 2015). Current clinical treatments include pain suppression, surgical intervention such as suturing (Rawson *et al* 2013), physiotherapy (Grisogono 1989), and cryotherapy (Knobloch *et al* 2007). Surgical procedures report high failure rates; for example, 35%–68% of rotor cuff surgeries fail every year due to formation of poor quality degenerative tendon unable to bear joint forces during rehabilitation after surgery (Schlegel *et al* 2006). Surgical procedures can also cause iatrogenic tendon defects while extracting tendon grafts for anterior cruciate ligament (ACL) substitutes (Cheng *et al* 2019).

Current strategies to enhance tendon repair, such as tissue engineering, cell-based therapies, and gene-based therapies, integrate scaffolds with appropriate levels of biochemical agents and cells to create a tissue that is similar to native tendon (Peach *et al* 2017). Engineered tissue has better mechanical properties than fibrous scar tissue, including Young's modulus, tensile strength, and maximum load bearing capacity (González-Quevedo *et al* 2018). Numerous scaffolds

such as nanofibrous matrices made from various biomaterials (Peach *et al* 2012, Jaiswal *et al* 2015, Manoukian *et al* 2017) or new biochemical factors have been explored to generate tendon tissues *in vitro* and in pre-clinical animal models (Ramos *et al* 2019). However, the factors affecting functionality are more basic than macroscale cell-biomaterial interactions. Shearn *et al* point to the need for more clinically relevant animal models and deeper understanding of the basic biological and mechanical features of tendon tissue (2011). Considering the major function of tendon, it is important to apply mechanobiology to study tendon regeneration processes at the cellular level. *In vitro* and *in vivo* joint immobilization studies have shown a correlation between tendon development and application of load. For example, one study has shown that steady application of load helps the development of hierarchical tendon fibril structure (Galloway *et al* 2013). This knowledge can be combined with engineering principles to develop bioreactors that can facilitate cellular exercise and create functional tendon tissue. This review details the developmental biology of tendon tissue, composition, physical properties, and its mechanosensory organelles. A particular emphasis is also made on the use of bioreactors in tendon tissue engineering (TTE) and related characterization.

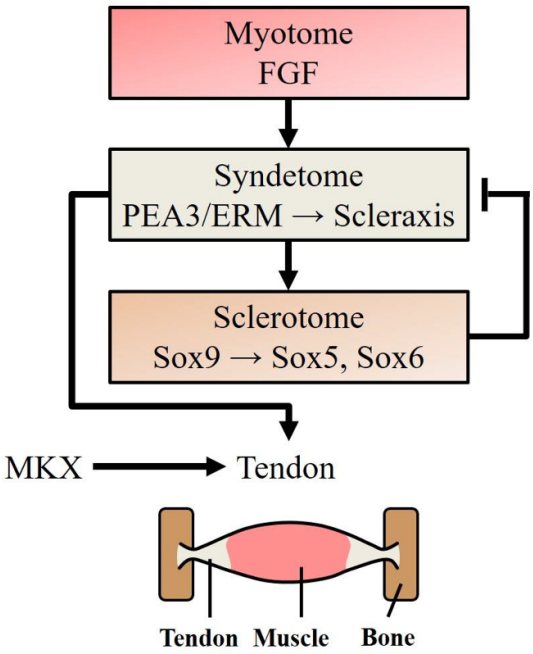

Figure 1. FGF pathway from myotome induces syndetome cells into tendon. This process is inhibited by Sox5 and Sox6, synthesized by developing cartilage cells in the sclerotome. Therefore, it is important to understand the inhibitory pathway during stem cell differentiation into the tenogenic lineage or co-culture between stem cells and tenocytes.

2. Tendon developmental biology

Tendons originate from the somatic mesoderm as part of the axial musculoskeletal system. The somite is patterned by surrounding tissues and causes the differentiation of the somite into different compartments and tissues. Scleraxis (Scx) has been identified as the major helix-loop-helix transcription factor related to tendons, acting as a marker for tendon progenitor cells. Scx mRNA has been shown to be expressed in both embryonic progenitor cells and adult tenocytes (Schweitzer *et al* 2001). Using this gene as a marker, many other signaling patterns have been studied as key to development of tendon tissue.

The current model of tendon differentiation and development in the embryo is based on signaling gradients of multiple genes from an adjacent part of the somite. In the trunk, fibroblast growth factor 8 (FGF8) signaling from the myotome induces transcription factors polyoma enhancer activator protein 3(PEA3) and Ets related molecule (ERM) to activate Scx expression, which induces differentiation of cells in the sclerotome to become tendons (figure 1). Tendon development is suppressed by SRY-box transcription factors Sox5 and Sox6 synthesized by cartilage-developing cells in the sclerotome. These two factors inhibit Scx expression and prevent other adjacent tissues from differentiating into tendons, despite signaling from the myotome (Liu *et al* 2011). $Sox5^{-/-}$ and $Sox6^{-/-}$ double mutant mice exhibit retarded cartilage differentiation due to overexpression of Scx (Brent *et al* 2005).

The bone-tendon insertion region has been predicted to follow the segregation model by Blitz *et al*. Long bones emerge from two sets of progenitor cells. One of them forms the primary bone and the other forms the bone eminence which creates the attachment site for tendon. These progenitor cells are Sox9 and Scx-positive and regulated by bone morphogenic protein (BMP) and the transforming growth factor beta (TGF-β) pathway (Blitz *et al* 2013). In contrast, muscle-tendon junctions form by migration of myoblasts to the site of insertion where they interact with tendon progenitor cells. Multiple signals help in this migration such as thrombospondin (Tsp) in the extracellular matrix (ECM) which binds to myoblast integrin (Subramanian and Schilling 2015).

Limb tendon progenitors arise in a slightly different pathway. In the limb bud, tendon and muscle progenitors are not separated into distinct regions and develop in the absence of Sox5/6, Myogenic factor 5 (Myf5), and Myogenic transcription factor MyoD genes. In the limb, tendon development is dependent on signaling from the muscle and ectodermal tissue. FGFs in the limb are potentially the source of these signals (Hoffmann and Gross 2006). Another possibility is that TGF-β also regulates tendon development in the limbs, as their signaling pathways are expressed

in the tendon during embryonic development (Liu *et al* 2011). A study by Berthet *et al* shows TGF-β activated Smad3 regulates critical transcriptional regulator Scx and Mohawk (Mkx), a homeobox gene (2013).

Tendon development is dependent on production and differentiation of the characteristic tendon fibroblast, and secretion of ECM and proteoglycans by these cells. Collagen Type 1 alpha 1 (Col1a1) gene expression is positively regulated by Scx, however, absence of Scx can differentially affect various parts of the body. Murchison *et al* showed that $Scx^{-/-}$ mice do not show complete disappearance of Type 1 collagen. The collagen in force-transmitting tendons were more affected compared to anchoring tendons in the $Scx^{-/-}$ mutant (2007). These results indicate the role of additional regulators of the Col1a1 gene. Lejard *et al* indicated a combinatorial role of two transcription factors that regulate Col1a1 gene expression in mice. Tendon-specific element 1 (TSE 1) and tendon-specific element 2 (TSE 2) binding with Scx and nuclear factor of activated T-cells cytoplasmic (NFATc) transcription factors, respectively, regulate Col1a1 expression. Inhibition of nuclear translocation of NFATc significantly reduced Col1a1 gene expression confirming the role of two target elements in Col1a1 regulation. Mkx is also a potential regulator of collagen. This transcription factor plays a vital role in development, cell proliferation, and differentiation of cells. Hypoplastic tendons were generated in $Mkx^{-/-}$ mice with smaller collagen fibril diameter and down regulated Col1a1 gene expression (Ito *et al* 2010).

3. Structural characteristics of tendon

3.1. Composition of tendon

Tendon is primarily composed of water and collagen. About 55%–70% of the tendon is water, with some water molecules associated with proteoglycans. Proteoglycans are made of leucine-rich proteins, including cartilage oligomeric matrix protein, fibromodulin, decorin, and aggrecan. Different compositions of proteoglycans vary with the location of the tendon, specialized for the type of force that tendon primarily encounters. Typically, 60%–85% of the dry weight of a tendon is made up of Type I collagen with 5% Type III and Type V collagen. Type III collagen is localized to the connective tissue of the tendon, in the endotenon and epitenon, forming less organized fibrils. Type V is found at the core of Type I fibrils, regulating fibril growth. Trace amounts of other collagens, including types II, VI, IX, X, and XI, are found at the bone insertion site (Wang 2006). Tropocollagen molecules cross-link to form insoluble collagen aggregate molecules. In addition, about 2% of the dry weight is composed of elastin fibers to provide elasticity (Hoffmann and Gross 2006). Glycoproteins are also present in the ECM of the tendon, most notably tenascin-C, which interact with collagen fibrils to improve mechanical stability, and fibronectin, located on the surface of collagen (Elefteriou *et al* 2001).

The structural elements making the ECM in tendon tissue are produced by two types of cells characteristic to the tendon: tenoblasts and tenocytes, the elongated fibroblasts arranged in rows between collagen fibers. The fibroblasts are spindle-shaped and produce collagen, fibronectin, proteoglycans, and other ECM proteins. They communicate through gap junctions (McNeilly *et al* 1996), and are connected to the ECM via integrins, permitting the cells to sense mechanical stimuli from outside the cell (Robi *et al* 2013). These cells make up 90%–95% of cells in the tendon. The remaining cells are chondrocytes, vascular endothelial and smooth muscle cells, and synovial cells of the tendon sheath (Hoffmann and Gross 2006).

3.2. Organization

Tendons are organized hierarchically, beginning with tropocollagen proteins as the most basic organizational unit. Collagen intermolecularly bonds to form a triple helix structure, and interacts with other collagen molecules to form the larger collagen fibril. Bundles of fibrils weave together to form the collagen fibers. Fibers are bound together by the endotenon in progressively larger groups, forming the sub-fascicle and then the fascicle. Fascicles are bound together by the epitenon, which is then surrounded by a final layer of connective tissue, the paratenon (Liu *et al* 2011). Each unit of the hierarchy runs parallel to the long axis of tendon, supporting the tendon's tensile strength (Wang 2006). The hierarchical organization of tendon can be seen in figure 2(A). This type of organization appears as a 'crimp pattern,' wherein the relaxed tendon has a wavy shape when relaxed, but straightens out when under stress (figure 3) (Stouffer *et al* 1985).

Tendon forms unique junctions called enthesis and myotendinous junctions with bone and muscle, respectively, on either end. Enthesis can be classified as fibrous or fibrocartilaginous. Fibrous enthesis attaches tendon directly to the bone or via a layer of periosteum and are present at the diaphysis of long bone and vertebral column. Fibrocartilaginous enthesis is present at the joints of the long bone and can be divided into four zones: (1) tendon or ligament, (2) uncalcified fibrocartilage, (3) calcified fibrocartilage, and (4) subchondral bone. This junction can bear 4 times more load than tendon tissue. Myotendinous junctions are made by insertion of tendon collagen fibrils into myofibroblast recesses. This structure allows for tensile force transmission from the muscle to the tendon (Wang 2006).

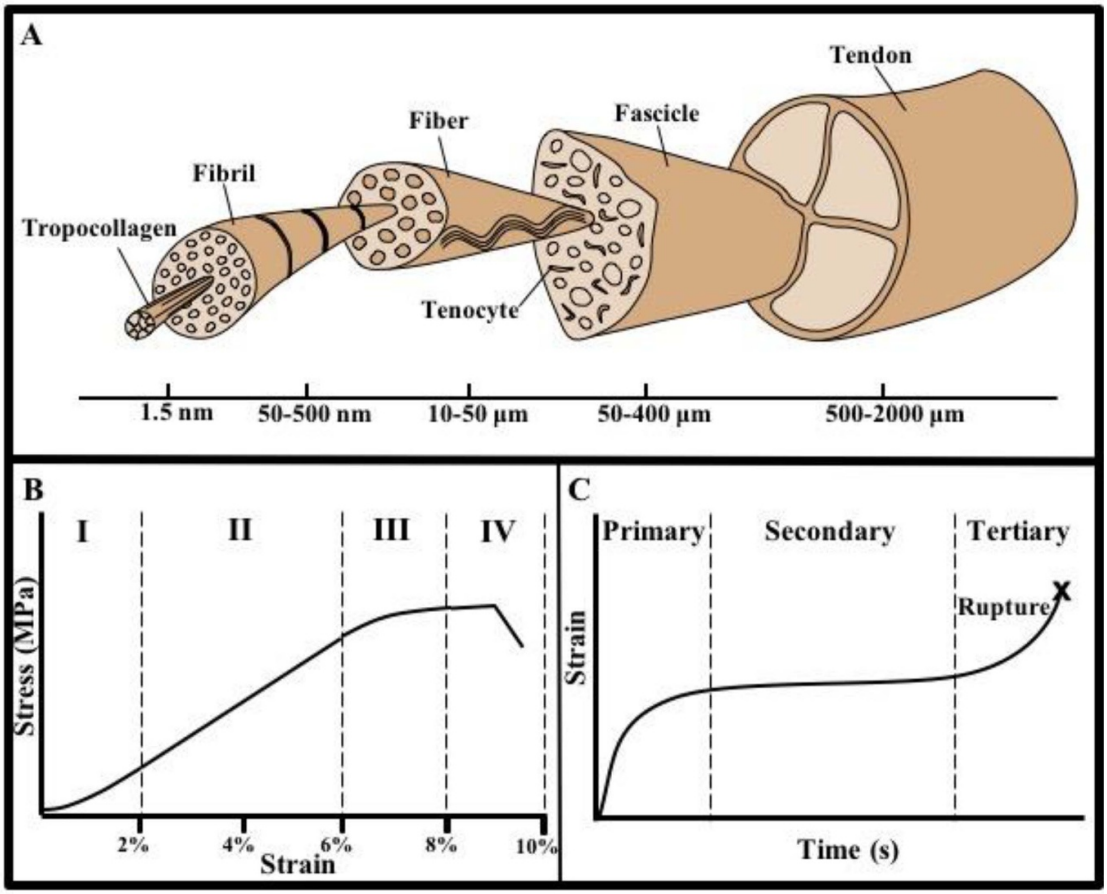

Figure 2. (A) Tendon structure: tropocollagen is the smallest building block that organizes into fibril, fiber, fascicle, and tendon tissue of varied length scales. (B) A typical stress–strain curve has four distinct regions characterized as Toe (I), Elastic (II), Microscopic Fracture (III), and Macroscopic Fracture (IV). Biomechanical considerations suggest benefits of tendon mechanical stimulation studies at the elastic region (strain at 2%–6%). (C) The creep curve represents the viscoelastic property of tendon which typically exhibits three distinct regions with rupture in the tertiary region. Therefore, TTE should focus on integrating structural, compositional, and mechanical aspects in the scaffold design.

3.2.1. Blood supply

The blood supply of tendon is provided from different sources that include the myotendinous junction, osteotendinous junction, and blood vessels of adjacent connective tissues (Liu *et al* 2011). Additionally, the blood vessels branch from the vessels in perimysium, periosteum, paratenon, and mesotenon. The myotendinous junction receives the blood supply through the vessels that penetrate the myotendinous junction, but it should be noted that only vessels from perimysium extend to the tendon. The middle part of the tendon possesses low vascularity, although it receives blood through the anterior surface from the paratenon area. The vessels from muscle bellies which extend to the endotenon, supply the blood for the third proximal part of the tendon. The lower part of the tendon receives blood by the vessels that supply the tendon bone junction, but because of the fibrocartilaginous layer between bone and tendon, the vessels do not have any communications. Vascularization decreases with maturation, with significantly more blood supply during development (O'Brien 1997, Doral *et al* 2010).

3.2.2. Nerve supply

The sensory nerves of tendon are provided by the overlying superficial nerves or adjacent deep nerves. The nerve receptors are classified into myelinated and unmyelinated types. Those which are sensitive to tension and pressure are considered myelinated and are located closer to the muscle. The unmyelinated types are responsible for sensing and transmitting pain (Lephart *et al* 1997, Ackermann *et al* 2001). There are four types of receptors sensitive to different stimuli that consist of (a) type I Ruffini corpuscles (sensitive to pressure and stretch), (b) type II Vater-Paccinian (sensitive to any movement), (c) type III Golgi tendon organs (mechanoreceptors), and (d) type IV free nerve endings (pain receptors) (O'Brien 1997).

3.2.3. Tendon metabolism

Similar to other collagen structured tissues, tendon has an active metabolism that responds to external factors. The metabolism rate of collagen is generally slow and has a balanced turnover rate, but will increase in response to external injury (O'Brien 1997). The rate of collagen degradation and synthesis

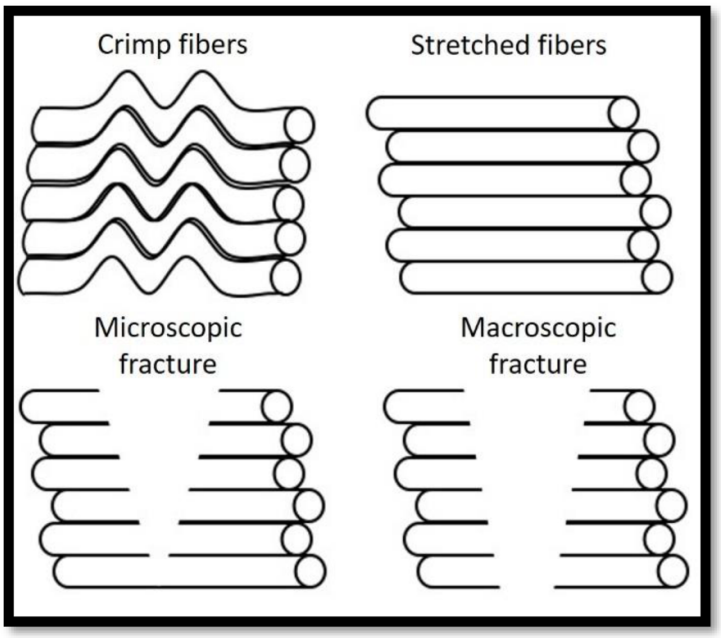

Figure 3. Tendon fibers show a crimp pattern in their relaxed state. When they are subjected to 2%–6% strain, they have a straightened morphology. Generally, a strain rate exceeding 6% will result in microscopic and macroscopic fractures in the tendon fibers.

in tendon is significantly reduced compared to bone. It is also impacted by training and inactivity. Tendon can loose its stiffness and collagen content with inactivity (Magnusson *et al* 2016). The procollagen in tendon is upregulated or down-regulated due to external movements while the extracellular collagen stays intact. In terms of oxygen consumption, tendon has 7.7 times and 10 times less oxygen consumption than skeletal muscle and liver tissue, respectively. The lower amount of oxygen consumption in tendon can be related to the lower collagen synthesis rate, as compared with muscle tissue (Vailas *et al* 1978, O'Brien 1997). High running performance is associated with lower oxygen consumption by Achilles tendon (Fletcher *et al* 2010).

4. Physical properties of tendon

4.1. Stress–strain curve

The tendon stress–strain curve (figure 2(B)) measures tensile strength and has three main regions: the toe region, the linear region, and the failure region (Robi *et al* 2013). The toe region is the un-crimping of the tendon's collagen fibril crimp pattern. This portion of the curve continues up to 2% strain, where the crimps have been fully extended (Wang 2006). The crimp pattern is variable between tendons located in different parts of the body and may have an effect on the tensile strength of the tendon, as shown in a study that compared the likelihood of rupture in different tendon tissues with different crimp angles. Here, it was demonstrated that fibers with small crimp angles fail before those with larger crimp angles (Wilmink *et al* 1992). The linear region follows, where the fibers respond linearly to the strain. If the strain is less than 4%, the tendon will return to its original length, back to its crimp pattern. However, between 4% and 8% strain, the collagen fibers will begin to slide past each other due to failure of the collagen triple helix cross-linking, leading to microscopic tearing of the fiber. As microscopic tears accumulate, the tendon undergoes an irreversible deformation. Beyond 8%–10% of strain, the tendon undergoes microscopic failure (Robi *et al* 2013). Further stretching may cause tendon rupture (David *et al* 1978).

However, it must be acknowledged that these boundaries of strain are variable between tendon locations. For example, in a 2003 study measuring the percent strain at which failure occurred in avian flexor tendons, the tendon held up 14% ramp-loading strain which was 3.5 times higher than previously reported strain tolerance (Devkota and Weinhold 2003). Another study testing the elastic strain limit of horse tendons showed a large variability of both the strain limit and stress curve of different tendons, varying along lines of individual test subjects and anatomical locations (Reyes *et al* 2014).

Mechanical properties of tendon can vary based on location of the tendon as well as age and gender. Human Achilles tendon is the strongest tendon and can bear a maximum load of 2258 $\pm$ 507 N, which is not significantly different from tendon with tendinopathy (Arya and Kulig 2010). In contrast, the maximum load for patellar tendon has been reported as

~5000 N for men and ~3000 N for women (O'Brien *et al* 2010). However, human tibialis anterior tendon can bear ~530 N isometric load at 2.5% strain (Maganaris and Paul 1999, 2000).

4.2. Viscoelasticity

The stress response of the tendon is not only dependent on the percent strain placed on it, but also the amount of time it is exposed to the strain. This concept, called viscoelasticity, is the effect of prolonged strain experienced by the tendon (Robi *et al* 2013). There are several viscoelastic properties that result from the tendency of tendons to respond differently to strain over time.

4.2.1. Creep

Creep is a viscoelastic property of the tendon that indicates the deformation under constant load. During primary creep, the tendon's crimp pattern straightens out (Hooley *et al* 1980). With prolonged exposure to load, the structures deform more and more, eventually reaching an asymptote (Hawkins *et al* 2009). The creep curve, measuring time versus strain with a constant load, can be broken down into primary, secondary, and tertiary creep regions, with the primary portion being the initial, rapid increase in stress, secondary being the asymptotic region, and tertiary being the rapid increase in strain resulting in failure (figure 2(C)). Primary creep is only a temporary deformation. However, tertiary creep will accumulate over time as a form of damage, eventually leading to a decrease of stiffness and strength in the tendon (Wang *et al* 1995). In an experiment measuring viscoelastic properties of the human Achilles tendon, 18 tendon specimens were examined *ex vivo*, undergoing constant stresses from 35 to 75 MPa. The resulting data produced a curve that is typical of the creep curve, and can be easily broken down into primary, secondary, and tertiary regions (Wren *et al* 2003). Creep may also be a good predictor of tendon strength. A study of creep and resultant rupture in wallaby tail tendons from the sacrocaudalis muscles revealed that creep responses are variable and dependent on the type of tendon, length, and temperature (Wang *et al* 1995).

4.2.2. Stress relaxation

Another phenomenon resulting from the viscoelastic properties of the tendon is stress relaxation, wherein stress is reduced over time due to constant deformation of the tendon. In models of stress relaxation, the two main variables are duration of stress and the overall amount of stress. Confirming this model, an experiment was performed on human patellar tendon and cruciate ligament. It was revealed that when separating the two variables, relative relaxation was affected by the time and stress variables under mechanical testing (Pioletti and Rakotomanana 2000). Another study examining stress relaxation responses of human patellar tendon revealed sections of tendons with larger surface areas relaxed at a significantly faster rate than those with smaller surface areas. This decrease in relaxation was non-linear, potentially suggesting the influence of connective tissue and structures other than the fascicle influencing the viscoelastic properties of the tendon (Atkinson *et al* 1999).

4.2.3. Hysteresis

Hysteresis is the difference in a material's stress response to strain when loading and unloading. The difference between these two curves is the amount of energy lost during loading (Robi *et al* 2013). The converse of hysteresis is the proportion of strain energy that is recovered elastically. Hysteresis values for tendons have been reported from 5% to 25%, a relatively low hysteresis, showing that most of the elastic energy stored during load application is preserved after unloading (Maganaris and Narici 2005). Human Achilles tendon maintains elastic properties over viscous properties with $5 \pm 2\%$ hysteresis with no effect of loading rate on stiffness (Peltonen *et al* 2013). In one study, adult human males had various leg tendons tested for hysteresis using a dynamometer footplate and electrodes. This resulted in a calculated hysteresis value of $19 \pm 3\%$ and a rebound resilience of $81 \pm 3\%$ (Maganaris and Paul 2000).

5. Mechanosensors of tendon

There are a number of mechanosensors, or biomolecules that respond to changes in mechanical force, that are integral to tendon function (figure 4). The ability of tendon tissue to respond to external forces has wide reaching effects, influencing tissue development and repair (Lavagnino *et al* 2015). These mechanosensors range from dynamic features such as primary cilia to transcription factors. However, while they are particularly salient for tendon tissue, none of the markers of tenocytes are particularly specific to tendon tissue during the *in vitro* testing stage (Lavagnino *et al* 2015). This may indicate that there are basic mechanisms of mechanosensing that are common amongst a greater variety of cell types.

5.1. Primary cilia

Primary cilia are mechanosensors common to many types of vertebrate cells. Cilia, extending as finger-like projections into the ECM, serve multiple functions. In tendon cells, a single primary cilium senses chemical and mechanical changes in the ECM, and further is able to effect gene expression. They are highly prevalent, having been observed in 64% of tenocytes in one study. Additionally, they are highly organized in the ECM, with orientation parallel to the collagen fibers of the tendon (Lavagnino *et al* 2015). In terms of mechanosensing, cilia have different physical responses to changes in mechanical force, requiring a certain level of deflection to elicit a response in

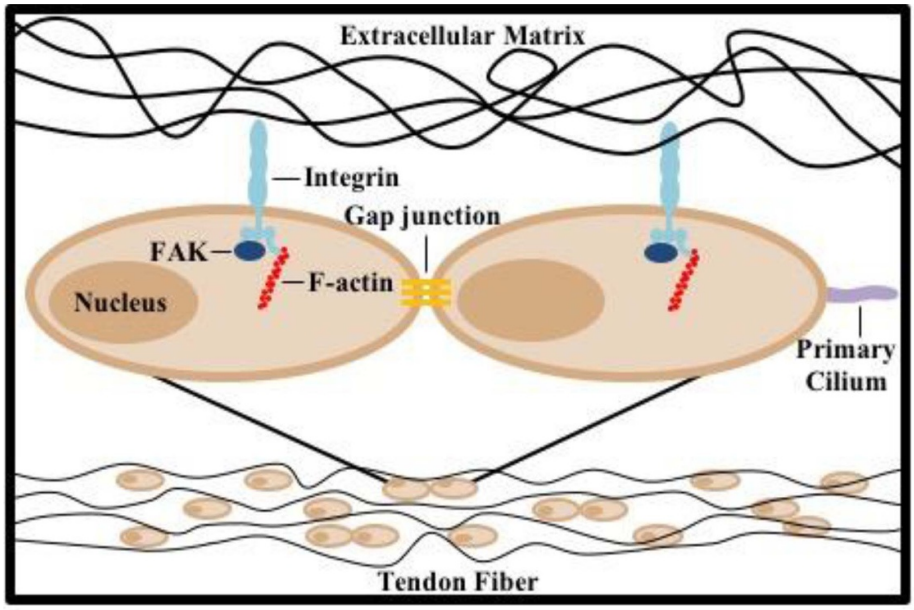

Figure 4. Mechanosensors within tenocytes sense micro level stress and strain exerted on both ECM and cells as a result of physical activity. This stimulation leads to increased levels of cell proliferation, matrix deposition, and differentiation. The commonly used mechanosensors for tendon regeneration include FAK, integrins, connexins, and cadherin proteins.

gene expression (Lavagnino *et al* 2011). The level of cilia deflection can be measured quantitatively and qualitatively by varying tensile loads. Four defection patterns of cilia include straight, curve, angle, and multi-angle. As tendons were loaded from 0% to 6% strain, cilia angles changed up to approximately 26% (Lavagnino *et al* 2011). The lack of mechanical strain also has an effect on cilia mechanical properties. Stress-deprivation of tendon cells has also been demonstrated to lengthen primary cilia after 24 h (Gardner *et al* 2011).

5.2. Focal adhesion kinase (FAK)

FAK also plays an important role in mechanosensing of cells, affecting tendon tissue differentiation. Some tenogenic gene expression may be partially regulated by FAK and transcription factors affected by FAK (Schiele *et al* 2013). It has been demonstrated in previous studies that dynamic strain may increase FAK phosphorylation in mesenchymal stem cells (MSCs), triggering reduction of collagen I and II production, and reducing production of tenascin-C and Scx (Xu *et al* 2011, 2012). It is also possible that FAK plays a role in tendon adhesion formation. In an *in vivo* study in chicken tendons, overexpression of the *FAK* gene induced by the injection of adenoviruses resulted in the formation of a thick layer of fibrous tissue as well as a significant increase in cell adhesions (Hoffmann and Gross 2006).

5.3. Integrins

Several integrins also play mechanosensing roles. Cell contraction occurs through transmission of contractile forces from actin filaments to the ECM using integrins as a bridge (Chrzanowska-Wodnicka and Burridge 1996). Integrins are able to facilitate mechanotransduction in two directions, one where signaling inside the cell alters integrin binding and cell adhesion, and another where ligands in the ECM interact with the integrin and cause a signal cascade (Wang 2006). In embryonic tendon cells, mechanosensing abilities may occur through contact between cell to ECM connections facilitated by integrins. Integrins transduce signals from the ECM to the cytoskeleton of the embryonic tendon progenitor cell (Schiele *et al* 2013). In one study, integrin $\alpha5\beta1$ was found in limb mesenchymal cells and later in connective tissues (Muschler and Horwitz 1991). Mesenchymal cells expressing integrin $\alpha11\beta1$ additionally seem to express in a similar pattern to Scx (Tiger *et al* 2001, Popova *et al* 2004). Integrins can form molecular complexes important to mechanosensing, in particular focal contacts, or streak-like, elongated adhesions. These adhesions tend to be associated with filament bundles (Rottner *et al* 1999, Zamir *et al* 2000). They may participate in adhesion-dependent signaling, evidenced by high levels of tyrosine-phosphorylated proteins found in focal contact adhesions (Yamada and Geiger 1997, Kreis and Vale 1999, Schoenwaelder and Burridge 1999). One study found that when mechanical force was applied *in vitro*, growth of focal contacts resulted (Riveline *et al* 2001). This process was determined to be dependent on the mDia1 signaling pathway (Riveline *et al* 2001).

5.4. Connexins

Gap junction proteins Connexins 32 and 43 have also been cited as important to mechanosensing, as they

facilitate cell to cell communication. Connexin 43 in particular links tendon longitudinally and laterally, while Connexin 32 links cells only longitudinally (Schiele *et al* 2013). Similarly to certain integrins, Connexins 32 and 43 are found in limb bud cells during embryonic development (Stanley *et al* 2007). Additionally, it has been demonstrated *in vivo* that downregulating Connexin 32 resulted in a lower level of stimulation of collagen synthesis in response to mechanical loading. In contrast, downregulating Connexin 43 had the opposite effect, increasing stimulation of collagen production (Waggett *et al* 2006). This suggests that both Connexin 32 and Connexin 43 have an effect on mechanosensing properties of cells.

5.5. Cadherins

Cadherins also participate in communication between cells, acting as adhesion proteins. Cell-to-cell connections like these play an important role in mechanotransduction (Leckband *et al* 2011). In tendons specifically, cadherin-11 has been identified as important in this function. In one study, cadherin-11 was downregulated in chick embryonic tendon tissue. This resulted in not only the loss of cell-to-cell contact, but also disruption of the ECM and collagen fibril organization (Richardson *et al* 2007). These results display the importance of cadherin-11 for both cell communication and organization.

6. Functional TTE

As an alternative to existing treatment and rehabilitation procedures, the field of tissue engineering relies on integration of scaffolds and cells to create a tissue *in vitro*. TTE approaches utilize both synthetic and natural polymer-derived scaffolds. Synthetic biodegradable polymers such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and co-polymers of these (poly lactic glycolic acid) (PLGA) have been used as scaffolding material. Knitted PLGA scaffolds seeded with bone marrow-derived stromal cells showed improvement in tendon healing in a 10 mm tendon defect in a rabbit model; The overall mechanical properties and collagen I and collagen III production improved (Ouyang *et al* 2003). Natural biomaterials such as collagen, silk, and polysaccharides such as chitosan have also been used for TTE for their bio-functional support of cell adhesion and proliferation. Knitted collagen-silk scaffold seeded with human embryonic stem cells differentiated into MSCs showed expression of tendon-specific markers and improved mechanical performance when implanted in a rat model (Chen *et al* 2010). Composite materials combining synthetic and natural biomaterials have also been used for TTE. A PLGA-collagen scaffold seeded with tendon-derived stem cells supported cell proliferation and production of neotendon after implanting the scaffold in nude mice (Xu *et al* 2014). Under *in vivo* mechanical stimulation, the scaffolds showed 1.5 times higher Young's modulus and ultimate stress after 12 weeks of implantation compared to a static condition. In order to replicate the natural fibrous morphology of tendons, scaffolds have been made using textile technology such as knitting (Chen *et al* 2010) and electrospinning. A layered electrospun matrix scaffold for rotor cuff repair compared tendon regeneration capacity of aligned and unaligned fiber matrices. The aligned matrices facilitated aligned collagen deposition which contributed to increased Young's modulus of the engineered tendon (Orr *et al* 2015). Recognizing the structure of the native tendon, Kew *et al* used a novel technique to create a collagen fascicle structure achieving failure stress of 25–45 MPa which is comparable to native tendon tissue (Orr *et al* 2015).

TTE primarily relies on tenocytes (Kryger *et al* 2007), MSCs (Bagnaninchi *et al* 2007), and dermal fibroblasts (Liu *et al* 2006) for seeding of scaffolds. These cells have shown positive results with respect to expression of tenogenic markers, and production of type 1 collagen and other tendon-related ECM components. Human embryonic stem cells differentiated into MSCs and modified to express Scx have shown enhanced mechanical properties and inhibition of adipogenic and chondrogenic markers when subjected to *in vivo* mechanical stimulation. Cells are able to translate the mechanical forces to relevant tissue regeneration components. A cellularized scaffold showed ~1.5 times higher mechanical properties such as stiffness, Young's Modulus, and failure force compared to acellular scaffold (Chen *et al* 2010). An electrospun yarn seeded with MSCs showed 73% higher Young's modulus compared to acellular yarn when cultured in dynamic conditions (Bosworth *et al* 2014). This indicates the importance of cellular interactions with scaffolds subjected to external forces in maintenance and repair of tendon tissue.

6.1. Biophysical conditioning using bioreactors

As tendon is an active tissue, its repair and maintenance are also dependent on cellular transduction of external forces using mechanosensors. In order to replicate the physio-chemical properties of native tendon tissue, engineers developed a dynamic environment and tools that mimic the stress and strain experienced by these tissues (Jaiswal *et al* 2017). Development of bioreactors has created physiologically relevant environments to produce functional tissues such as bone (Peroglio *et al* 2018), ligament (Lee *et al* 2010), and cartilage (Peroglio *et al* 2018). Use of a bioreactor has produced tendons with 1.7 times improved tensile force compared to unstimulated tendon (Wang *et al* 2015). When scaffolds laden with cells are subjected to stimulation, the various mechanosensors of the cells actively transcend the activity signal via signaling pathways to the nucleus

Table 1. Commercially available bioreactors used for TTE.

Type	Company	Product	Ref.
Biaxial Tension	CellScale	MCB1	(cellscale, accessed July 2019)
Compression: Hydrostatic Pressure	BISS Tissue Growth Technologies	CatiGen HP	(tissuegrowth, accessed July 2019)
Compression	BISS Tissue Growth Technologies	CartiGen	
	CellScale	MCTR	(cellscale, accessed July 2019)
	Flexcell International Corporation	FX-5000™ Compression System	(flexcellint, accessed July 2019)
	MATEsystems	MATE	(matesystems 2014, accessed July 2019)
	TA Instruments	ElectroForce 5500	(TAinstruments, accessed July 2019)
Compression & Tension	Ebers Medical	TC-3 & TC-3F Bioreactor	(EbersMedical, accessed July 2019)
	St3corp	Oscillatory Flow Bioreactor Drive System	(Corp, accessed July 2019)
	TA Instruments	BioDynamic 5100 & 5200	(TAinstruments, accessed July 2019)
Pulsating Pressure & Flow	BISS Tissue Growth Technologies	LumeGen	(tissuegrowth, accessed July 2019)
Rotary	Synthecon	CardioGen RCCMAX RCCMAX DUAL	(Synthecon, accessed July 2019)
Uniaxial Tension	ADMET	BioTense Bioreactor	(Admet, accessed July 2019)
	BISS Tissue Growth Technologies	DermiGen	(tissuegrowth, accessed July 2019)
	CellScale	LigaGen MCFX MCT6	(cellscale, accessed July 2019)
	Flexcell International Corporation	Flexcell® FX-5000™ Tension System Flexcell® FX-6000™ Tension System Flex Jr.™ Tension System Tissue Train® Culture System	(flexcellint, accessed July 2019)
	BISS Tissue Growth Technologies	LigaGen	(tissuegrowth, accessed July 2019)

which in turn leads to cell proliferation or differentiation (Lee *et al* 2010). Table 1 gives a list of commercially available bioreactors used for TTE.

Bioreactors can be used in four main applications: (1) maintaining the condition of organs *ex vivo*, (2) preparing cells before transplantation, (3) simple pathway studies, and (4) growing tissues *in vitro* (Youngstrom and Barrett 2016). Lee *et al* designed a cyclic strain bioreactor to study the mechanical properties and maintenance of decellularized tendon (2013). The physical strength of the tendon improved significantly after being subjected to tensile and torsional deformation. Further, tendon homeostasis was studied in rabbit Achilles tendon by Xu *et al* using a programmable mechanical stimulation device (2015). Their results showed improved collagen fiber orientation and reduced type III collagen expression after cyclic mechanical stimulation. In order to use a bioreactor as a cell priming device, Riboh *et al* studied mechanical manipulation of cultured tendon cells in scenarios with different amplitude, frequency, and on/off ratio to target the ideal parameters for cell proliferation, collagen production, and tendon morphology (2008). An intermittent cyclic strain improved cell proliferation, promoted collagen I production, and maintained tenocyte morphology better than continuous cyclic strain or no strain. Rabbit flexor tendon reseeded with live cells have shown improvements in mechanical properties along with an increase in tendon type cells after being cultured in a bioreactor (Thorfinn *et al* 2012). Bioreactors can also be used to study basic pathways and grow replacement tissues using biomaterial scaffolds. Three-dimensional (3D) scaffolds seeded with stem cells are subjected to cyclic tensile strain. These scaffolds show increased tendon associated markers, such as tenascin-C, tenomodulin, and Scx, compared to unstimulated control cases. The stimulated samples also have higher type I collagen expression (Xu *et al* 2015).

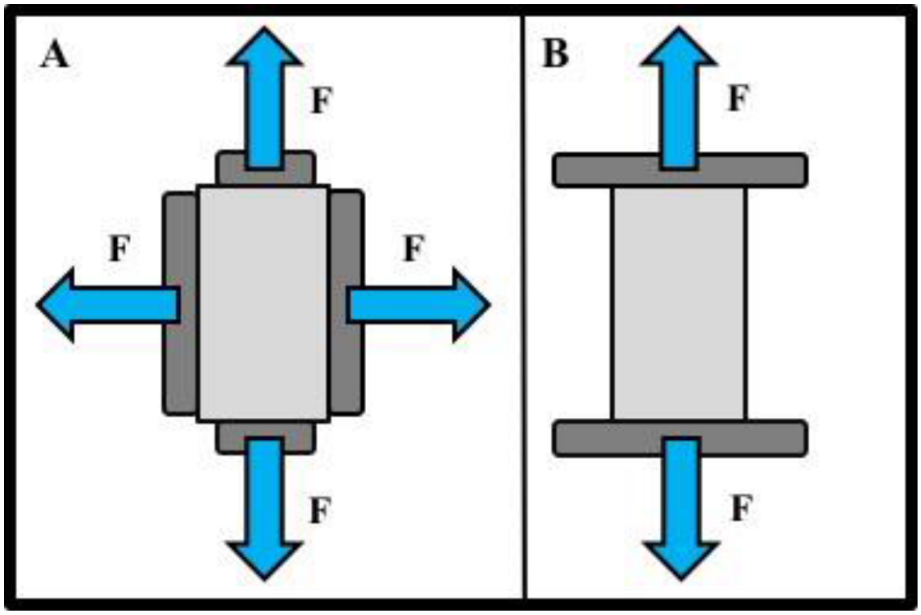

Figure 5. Bioreactor configurations to apply (A) biaxial or (B) uniaxial stimulation to scaffolds. The scaffolds seeded with cells are held between clamps with application of either bidirectional or unidirectional force F to study tissue development. Uniaxial bioreactors are popularly used for engineering tendon and ligament tissue with applied strains in the elastic region.

6.2. Types of tendon bioreactors

Bioreactors used specifically for TTE can be categorized based on the type of sample, stimulation regime, culture chambers, and sample size.

6.2.1. Uniaxial and biaxial

Bioreactors made for tendon are focused on uniaxial tensile and compression loading which is parallel or transverse to the longitudinal orientation of tendon fibers (figure 5(A)). Commercially available uniaxial bioreactor models from CellScale are actively being used for tendon research to compare phenotypical and genotype expression of tenocytes, dermal cells, and MSCs (Gaspar *et al* 2017). Typically, tenocytes seeded on parallel or yarn-based electrospun fibrous scaffolds orient themselves parallel to the direction of the applied strain (Wu *et al* 2017). The CellScale uniaxial stimulation device has also been used to study bone marrow stromal cell interactions with tendon constructs for rotator cuff repair (Qin *et al* 2015). In addition to commercially available devices, there are custom-made uniaxial stimulation devices that cater to the particular research need, such as Ligagen-L30-4C (DynaGen Systems) used for flexor tendon (Saber *et al* 2010), and programmable logic-controlled Achilles tendon bioreactor (Zhang and Wang 2013).

Generally, biaxial bioreactors are designed for tissues such as bone, skin, and muscle. Ravichandran *et al* used a biaxial bioreactor that mimics fetal rotation in order to explore the mechanical strain during early development (figure 5(B)). This bioreactor rotates along the *x*-axis and *z*-axis, and can apply cyclic compression to test bone constructs (2018). A biaxial mechanical loading device from CellScale has been used to study soft tissues cells such as endothelial cells, fibroblasts, osteoblasts, and MSCs (Wang *et al* 2018a). Even though previously conducted tendon studies focus on uniaxial stimulation, Szczesny *et al* used biaxial testing to evaluate the correlation between collagen fiber angle distribution and human supraspinatus tendon mechanics in order to understand more about the structure-function relationships in fibrous musculoskeletal tissue (2012).

Both uniaxial and biaxial bioreactors stimulate mechanosensors such as integrins. Tendon-derived stem cells (TDSCs) can differentiate into different lineages when stimulated by uniaxial or biaxial bioreactors. Uniaxial loading phosphorylates protein kinase B (PKB) forming tenogenic or osteogenic cells whereas biaxial loading phosphorylates extracellular signal-regulating kinase (ERK) signaling producing adipogenic or osteogenic cells (Wang *et al* 2018b). Tendon differentiation markers such as Scx, Mkx, Col1a1, and tenomodulin can be upregulated by uniaxial loading and inhibited by biaxial loading. This shows that mechanosensing signaling pathway response is different during uniaxial and biaxial loading for TDSCs. Confirming the role of uniaxial loading in tendon development, tendon sheath differentiation marker Tubulin polymerization-promoting protein family member 3 (Tppp3) was significantly upregulated after cyclic uniaxial loading (Xu *et al* 2015). Tppp3 is linked to activation of hedgehog signaling which is regulated by patched1 at primary cilia, a tendon mechanosensor (Rohatgi *et al* 2007, Wang *et al* 2017).

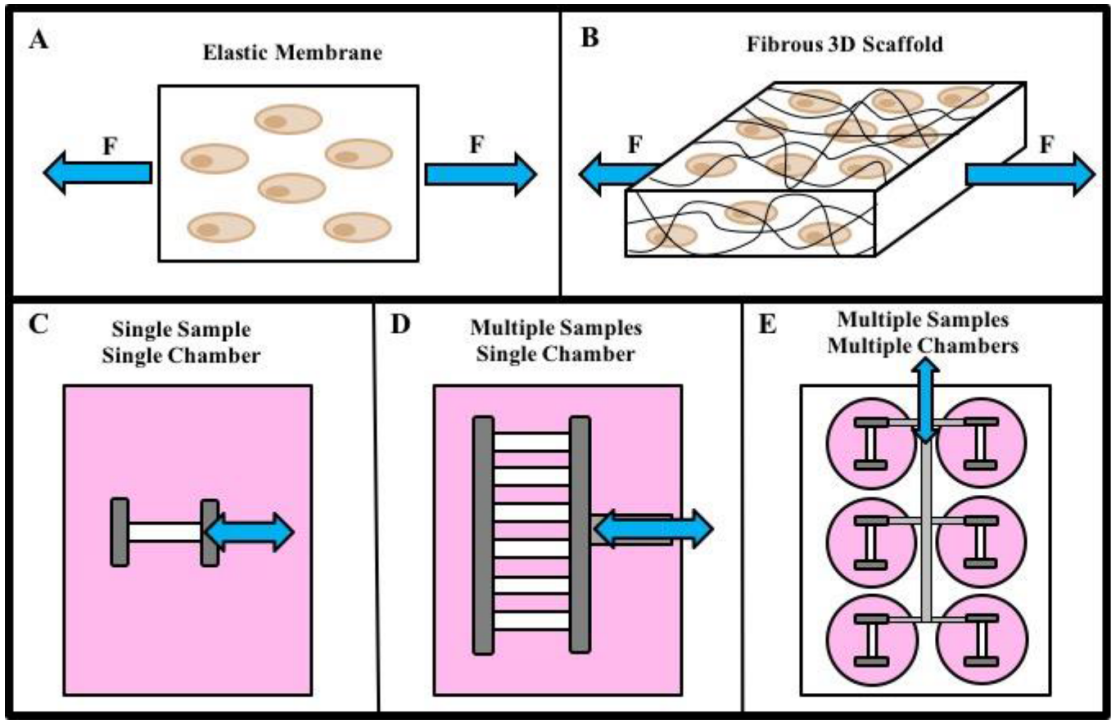

Figure 6. Types of tendon bioreactors stimulating (A) a cell monolayer on elastic membrane and (B) cells in a 3D fibrous scaffold environment. Chamber-based classes of bioreactors are: (C) single chamber with one sample, (D) single chamber with multiple samples, and (E) multiple chambers with multiple samples. All these bioreactors apply uniaxial strain to the samples as indicated by direction of force F application arrows (blue). Mechanical stimulation using bioreactors has shown 2D cells as well as cells seeded on 3D scaffolds respond to unidirectional strain to facilitate cell differentiation.

6.2.2. 2D and 3D samples

Tissue engineering studies are focused on understanding cellular mechanics and their integration into 3D scaffolds to form a tissue. Cellular studies are conducted in a bioreactor by seeding cells on a flexible membrane (figure 6(A)). A linear actuator or a negative pressure system can be used to stretch the cells. Such a dynamic cell monolayer study has been conducted using a commercially available stimulator from Flexcell (Matheson *et al* 2006). The Flexcell six-well culture plate consists of a silicone membrane with high affinity cell attachment. This system has been used to study tendon cell-cell interactions and actin filament alignment when subjected to biaxial strain. Loaded cells show stronger cell-cell attachment and an organized cytoskeleton that responds to the direction of the strain (Ralphs *et al* 2002). A cell source optimization study for flexor TTE was conducted using Flexcell showing increased collagen production for stimulated bone marrow-derived stem cells compared to sheath fibroblast and adipose-derived stem cells (Riboh *et al* 2008). Even though 2D sample bioreactors are beneficial for studying cellular behavior, they cannot be used to develop tendon 3D tissue. Altman *et al* designed one of the first mechanical stimulators that could work with 3D samples (2002). Following this study, many commercial bioreactors were developed to accommodate 3D samples (figure 6(B)). The complex cell–cell and cell-matrix interactions on a 3D electrospun polycaprolactone (PCL) fibrous yarn showed differentiation of MSCs to tendon lineage when loaded for 21 d producing 30 μm thick cell sheet around the yarn when compared to static culture (Bosworth *et al* 2014). Likewise, certain models of commercially available bioreactors, such as Flexcell and TA instruments, can mechanically stimulate 3D tendon samples such as explanted tissue and cell seeded scaffolds made from natural or synthetic biomaterials. Flexcell's Flexercell strain unit can be used to uniaxially stimulate collagen gel laden with tendon cells in a six-well plate where cells reorganize the collagen fibrils, align in the direction of the mechanical load, and produce tissue with ultimate tensile strength 3-fold higher than unstimulated sample (Garvin *et al* 2003).

Activation of mechanosensors can be different in 2D and 3D constructs. Connexin 43 is upregulated in 2D uniaxial and biaxial loading, but it is inhibited in uniaxial-loaded 3D constructs (Wang *et al* 2018b). Uniaxial loading of 3D constructs has shown at least 8 times higher Col1a1 expression and twice as high Young's Modulus for tendon fibroblasts compared to 2D culture (Testa *et al* 2017). 2D and 3D cultures have been compared using FlexCell system which showed 6 times more Scx expression for uniaxially loaded 3D constructs compared to 2D-cultured human adipose stem cells (Yang *et al* 2013). Upregulation of Scx expression can be linked to activation

of the TGFβ2 signaling pathway (Barnette *et al* 2013). TGFβ cell surface receptors sense external mechanical forces to activate the pathway which in turn upregulates Scx. Scx expression has also been linked to promoting adult tenocyte mechanical sensing capacity via integrins. Scx knockdown cells show reduced expression of proteins associated with focal adhesion (Nichols *et al* 2018). Thus, higher levels of Scx due to uniaxial loading of 3D cultures not only promotes tenogenic differentiation but also supports mechanosensing activity of tendon.

6.2.3. Loading regimes

Loading regimes for tendon stimulation are guided by tendon anisotropic behavior and fiber orientation. As described in the previous section, tendon exhibits a nonlinear toe zone up to 2% strain and a linear region from about 2% and 6% strain (figure 2(B)), and undergoes micro and macro-fracture prior to ultimate rupture (Wang *et al* 2018a). Parameters such as percentage strain, amplitude, frequency, duration, on/off cycle, and loading direction with respect to fiber orientation are to be considered when planning the loading regime for tendon. Most of the previous studies have chosen a strain rate between 4% and 8% strain, as that falls within the linear elastic region (Butler *et al* 1978). Studies comparing different strain rates show the presence of an optimum condition that gives maximum cell survival rate and higher expression of tendon markers. For example, 9% strain can cause damage to the cells compared to 6% and 3% strain (Wang *et al* 2013). Comparative studies show lower strain amplitude applied to tendon-derived stem cells can induce tenogenic differentiation, whereas higher strains can induce osteogenic, adipogenic, or chondrogenic differentiation (Peroglio *et al* 2018). Likewise, there are other reported variabilities in the choice of parameters that should be optimized, such as frequency and duration (Riboh *et al* 2008).

Table 2 lists the various loading regimes and their outcomes. Considering a need for an optimization study, Riboh *et al* studied the variations of these parameters and came to multiple conclusions regarding their effects on tendon regeneration. Cellular proliferation, collagen production, and tendon morphology were compared. The study included combinations of two strain amplitudes (4% and 8%), three frequencies (0, 1 and 0.1 Hz), and four on/off ratios (static, always on, 1:2, 1:5). The outcomes suggest that a continuous cyclic strain inhibited cell proliferation and increased collagen production, while intermittent cyclic strain (ICS) increased cell proliferation, cell alignment, and nuclear elongation. Other studies confirmed that intermittent cyclic strain yields the best results (Xu *et al* 2015). Even though most regimes result in some degree of cell differentiation, collagen secretion, and expression of tendon markers, there is no conclusive loading regime that can be used universally for tendon regeneration.

Physical stimulation of cells can affect the proteins involved in mechanosensing such as FAK, vinculin, and integrins. As a downstream response, intracellular levels of calcium can change due to varied loading regimes. In 2005, Wall and Banes observed increased intracellular calcium in rat tail tendon cells when subjected to 1%–6% strain (2005). Parameters such as magnitude and hours of loading cycle can vary intracellular calcium levels due to loading of a combination of mechanosensors. Human tenocytes showed the highest level of intracellular calcium when subjected to 12% strain for 8 and 12 h compared to a 4 h loading cycle (Chen *et al* 2015). A recent study showed the role of stretch-activated calcium channels (SACC) in tenogenic differentiation of human MSCs (Nam *et al* 2017). Tenogenic differentiation markers decreased when SACCs were blocked and stimulated for 6, 12, or 24 h.

6.2.4. Number of samples and chambers

Sample number and testing of multiple conditions can be crucial specifications for designing or selecting a bioreactor. A single chamber bioreactor, stimulating one sample at a time, results in wasted time and resources to derive statistically significant data since most tendon regeneration studies last 7–21 d (figure 6(C)) (Woon *et al* 2011). In order to run parallel experiments, researchers have to set up multiple expensive bioreactors. Typical cost of a simple commercial bioreactor ranges 8000–10 000 USD. This situation can be circumvented by creating bioreactors with multiple chambers and multiple sample holding capacity. Single chamber bioreactors with multiple sample holders are available both commercially (CellScale, Mechano-bioreactor MCT6) and have been custom designed for tendon (figure 6(D)) (Angelidis *et al* 2010, Wang *et al* 2013). These bioreactors reduce sample to sample variability during a single experimental duration. However, these devices are incapable of testing multiple treatment conditions in a single device.

Recently, bioreactors with multiple chambers have become the norm, especially for uniaxial models. A few commercial bioreactors have found widespread utility for the study of tendon. Flexcell International Corporation designed the Flexcell tension systems which have been used in multiple studies. The design utilizes vacuum pressure to apply cyclic or static strain to flexible culture plates. In addition, this bioreactor allows for custom programmable waveform, amplitude, and frequency variations. Another popular product with multiple chambers is the CellScale MechanoCulture FX (MCFX). The design includes a single-use, flexible silicone well plate with 16 individual chambers. After the cells adhere to the plate, the user can specify the protocol and mechanically stress the cells. This stretchable membrane is an

Table 2. Loading regimes used for 3D scaffold stimulation in TTE. ICS: intermittent cyclic strain, CCS: continuous cyclic strain.

Routine	% Strain	Frequency (Hz)	On:Off Ratio (Hours)	Proliferation	Collagen Production	Cellular Alignment	Ref
Control	0	0	Static				
ICS	4	0.1	1:2	Slight increase	Slight increase	Increase	Riboh *et al* (2008)
ICS	4	0.1	1:5	Increase	Increase	Increase	
CCS	8	1	Always on	Decrease	Increase	Increase	
ICS	3	0.33	1:23	N/A	Increase	Increase	Youngstrom *et al* (2015)
ICS	5	0.33	1:23	N/A	Slight increase	Increase	
CCS	10	0.1	Always on	No change	Increase	Increase	Huisman *et al* (2014)
ICS	3	0.25	8:16	Increase	Increase	Increase	Wang *et al* (2013)
ICS	6	0.25	8:16	Increase	Highest	Increase	
ICS	9	0.25	8:16	Decrease	Increase	Decrease	

example of 2D dynamic culture. Again, this allows for multiple samples to be modeled and strained at once. In the academic realm, a few research groups have designed their own custom bioreactors for specific studies. The advantage in designing a bioreactor is the customizability and use of 3D samples. For instance, Lee *et al* effectively designed and utilized a custom 10-chamber bioreactor for their study. This bioreactor was able to simultaneously apply equal uniaxial cyclic tensile strain to 10 separate scaffolds (figure 6(E)).

Thus, tendon bioreactors are limited by number of samples, as well as cost incurred towards each unit due to development of a humidity safe compartment for electronics or creating ambient cell survival conditions. Future work in bioreactor engineering should focus on creating a user-friendly yet cost-effective multiple-chamber, multiple-sample holding system. Since biological studies require testing multiple conditions with a statistically significant sample number, it is not sufficient to work with expensive single-sample bioreactors.

7. Future considerations

Tendon regeneration has heavily relied on using growth factor supplements for cellular differentiation of MSCs into the tenogenic lineage. However, cell-cell and cell-ECM interactions are being explored for cellular differentiation via inclusion of co-culture and mechano-stimulation. The effect of scaffold-related factors such as material topography (Lee *et al* 2013), stiffness (Vijayavenkataraman *et al* 2017), and chemical composition (Chen *et al* 2009) have shown the ability of cells to sense physical properties of substrates. Likewise, co-culture of two different cell types has shown changes in proliferation and differentiation patterns. Luo *et al* studied interaction of tenocytes with bone marrow-derived MSCs by using indirect co-culture. Results showed an increase in proliferation and up-regulation of tendon/ligament-related genes after 14 d of culture (2009). A comparative study conducted by Kraus *et al* showed a 4-fold up-regulation of tenascin C expression in adipose-derived stem cells when co-cultured directly with tenocytes. This study also showed that direct co-culture is more effective than indirect co-culture with only a 2.5-fold increase in tenascin C compared to control (2013). Exchange of cellular content between primary tenocytes and MSCs was confirmed by Schneider *et al* by using membrane markers and, endocytosis and exocytosis of gold nanoparticles. Results from transmission electron microscopy images showed clear exchange of cellular components between MSCs and tenocytes (2011). This study confirmed strong tenogenic induction capability of tenocytes when seeded with MSCs at even lower tenocyte percentage in a 3D culture. Thus, a combined effect of cell-cell communication in a 3D co-culture and mechano-sensing of forces transduced from the microenvironment may be a pathway to creating a functional tendon tissue without the need for growth factor supplements.

Our ongoing efforts are focused on the design and validation of a simple bioreactor that enables multiple sample and condition testing using regular tissue culture supplies and incubator. In that direction, we have recently developed a user-friendly multi-chamber, multi-sample holding bioreactor for uniaxial mechanical stimulation of tendon tissue and biomaterial scaffolds (Zolnoski *et al* 2019). This bioreactor has a multi-arm design that fits a commercially available six-well plate and supports simultaneous studies for statically relevant data. By using equipment available in a standard cell culture laboratory, it is a cost-effective dynamic culture platform.

8. Conclusion

A successful TTE strategy should integrate tissue-mimicking scaffolds, suitable cell sources, biochemical signals, and biomechanical considerations to replicate native tissue microenvironments. An in-depth knowledge of tendon development in the embryonic state and its relation to superior mechanical properties of native tendon can support future studies. Mechanosensors such as integrins, connexins, and primary cilia respond to amplitude and directionality of force. Engineered environments created in a bioreactor have the potential to mimic the dynamic nature of a joint that tendon mechanosensors experience. They can be designed to create functional tendon or study biological processes. These dynamic cultures using bioreactors can provide animal alternatives to screen bioactive molecules towards tendon/ligament regeneration. Future research entails using optimized biological and mechanical-stimulation studies to regenerate tendons such that they may be produced as an implantable product.

Acknowledgments

The authors acknowledge funding support from the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health (R01EB020640) and the Connecticut Regenerative Medicine Research Fund (15-RMBUCHC-08). We thank Dr Christopher Bonin for his editing support.

ORCID iDs

Devina Jaiswal https://orcid.org/0000-0003-1699-2043
Laurie Yousman https://orcid.org/0000-0003-2492-2408
Sangamesh G Kumbar https://orcid.org/0000-0001-7672-4783

References

Ackermann P W, Li J, Finn A, Ahmed M and Kreicbergs A 2001 Autonomic innervation of tendons, ligaments and joint capsules. A morphologic and quantitative study in the rat *J. Orthop. Res.* **19** 372–8

ADMET Universal Testing Systems, Fixtures, and Accessories www.admet.com (accessed July 2019)

Altman G, Horan R, Martin I, Farhadi J, Stark P, Volloch V, Vunjak-Novakovic G, Richmond J and Kaplan D L 2002 Cell differentiation by mechanical stress *FASEB J.* **16** 270–2

Angelidis I K, Thorfinn J, Connolly I D, Lindsey D, Pham H M and Chang J 2010 Tissue engineering of flexor tendons: the effect of a tissue bioreactor on adipoderived stem cell–seeded and fibroblast-seeded tendon constructs *J. Hand Surg. Am.* **35** 1466–72

Arya S and Kulig K 2010 Tendinopathy alters mechanical and material properties of the Achilles tendon *J. Appl. Physiol.* **108** 670–5

Atkinson T S, Ewers B J and Haut R C 1999 The tensile and stress relaxation responses of human patellar tendon varies with specimen cross-sectional area *J. Biomech.* **32** 907–14

Bagnaninchi P-O, Yang Y, El Haj A J and Maffulli N 2007 Tissue engineering for tendon repair *Br. J. Sports Med.* **41** e10

Barnette D N, Hulin A, Ahmed A I, Colige A C, Azhar M and Lincoln J 2013 Tgfβ-Smad and MAPK signaling mediate scleraxis and proteoglycan expression in heart valves *J. Mol. Cell. Cardiol.* **65** 137–46

Berthet E, Chen C, Butcher K, Schneider R A, Alliston T and Amirtharajah M 2013 Smad3 binds Scleraxis and Mohawk and regulates tendon matrix organization *J. Orthop. Res.* **31** 1475–83

BISS Tissue Growth Technologies www.tissuegrowth.com (accessed July 2019)

Blitz E, Sharir A, Akiyama H and Zelzer E 2013 Tendon-bone attachment unit is formed modularly by a distinct pool of Scx-and Sox9-positive progenitors *Development* **140** 2680–90

Bosworth L, Rathbone S, Bradley R and Cartmell S 2014 Dynamic loading of electrospun yarns guides mesenchymal stem cells towards a tendon lineage *J. Mech. Behav. Biomed. Mater.* **39** 175–83

Brent A E, Braun T and Tabin C J 2005 Genetic analysis of interactions between the somitic muscle, cartilage and tendon cell lineages during mouse development *Development* **132** 515–28

Butler D, Grood E, Noyes F and Zernicke R 1978 Biomechanics of ligaments and tendons *Exercise Sport Sci. Rev.* **6** 125–82

CellScale Biomaterials Testing mechanical test systems and bioreactors www.cellscale.com (accessed July 2019)

Chen J, Xu J, Wang A and Zheng M 2009 Scaffolds for tendon and ligament repair: review of the efficacy of commercial products *Expert Rev. Med. Devices* **6** 61–73

Chen J L, Yin Z, Shen W L, Chen X, Heng B C, Zou X H and Ouyang H W 2010 Efficacy of hESC-MSCs in knitted silk-collagen scaffold for tendon tissue engineering and their roles *Biomaterials* **31** 9438–51

Chen W, Deng Y, Zhang J and Tang K 2015 Uniaxial repetitive mechanical overloading induces influx of extracellular calcium and cytoskeleton disruption in human tenocytes *Cell Tissue Res.* **359** 577–87

Cheng B, Tian J, Peng Y and Fu X 2019 Iatrogenic wounds: a common but often overlooked problem *Burns Trauma* **7** s41038-019

Chrzanowska-Wodnicka M and Burridge K 1996 Rho-stimulated contractility drives the formation of stress fibers and focal adhesions *J. Cell Biol.* **133** 1403–15

Corp S Cell Stimulation 3D www.st3corp.com/bioreactor-drive-systems.cfm (accessed July 2019)

David L, Grood E S, Noyes F R and Zernicke R E 1978 Biomechanics of ligaments and tendons *Exercise Sport Sci. Rev.* **6** 125–82

Devkota A C and Weinhold P S 2003 Mechanical response of tendon subsequent to ramp loading to varying strain limits *Clin. Biomech.* **18** 969–74

Doral M N, Alam M, Bozkurt M, Turhan E, Atay O A, Dönmez G and Maffulli N 2010 Functional anatomy of the Achilles tendon *Knee Surg. Sports Traumatol. Arthrosc.* **18** 638–43

EBERS Medical Bioreactors for tissue engineering research www.ebersmedical.com (accessed July 2019)

Elefteriou F, Exposito J-Y, Garrone R and Lethias C 2001 Binding of tenascin-X to decorin *FEBS Lett.* **495** 44–47

Fletcher J R, Esau S P and MacIntosh B R 2010 Changes in tendon stiffness and running economy in highly trained distance runners *Eur. J. Appl. Physiol.* **110** 1037–46

Flexcell® International Corporation www.flexcellint.com/products (accessed July 2019)

Galloway M T, Lalley A L and Shearn J T 2013 The role of mechanical loading in tendon development, maintenance, injury, and repair *J. Bone Joint Surg. Am.* **95** 1620

Gardner K, Arnoczky S P and Lavagnino M 2011 Effect of *in vitro* stress-deprivation and cyclic loading on the length of tendon cell cilia *in situ J. Orthop. Res.* **29** 582–7

Garvin J, Qi J, Maloney M and Banes A J 2003 Novel system for engineering bioartificial tendons and application of mechanical load *Tissue Eng.* **9** 967–79

Gaspar D, Pandit A and Zeugolis D 2017 Tenogenic phenotype maintenance and differentiation using macromolecular crowding and mechanical loading *Orthop. Proc.* **99** 39

González-Quevedo D, Martínez-Medina I, Campos A, Campos F and Carriel V 2018 Tissue engineering strategies for the treatment of tendon injuries: a systematic review and meta-analysis of animal models *Bone Joint Res.* **7** 318–24

Grisogono V 1989 Physiotherapy treatment for Achilles tendon injuries *Physiotherapy* **75** 562–72

Hawkins D, Lum C, Gaydos D and Dunning R 2009 Dynamic creep and pre-conditioning of the Achilles tendon *in-vivo J. Biomech.* **42** 2813–17

Hoffmann A and Gross G 2006 Tendon and ligament engineering: from cell biology to *in vivo* application **1** 563–74

Hooley C, McCrum N and Cohen R 1980 The viscoelastic deformation of tendon *J. Biomech.* **13** 521–8

Huisman E, Lu A, McCormack R G and Scott A 2014 Enhanced collagen type I synthesis by human tenocytes subjected to periodic *in vitro* mechanical stimulation *BMC Musculoskelet. Disord.* **15** 386

Ito Y, Toriuchi N, Yoshitaka T, Ueno-Kudoh H, Sato T, Yokoyama S, Nishida K, Akimoto T, Takahashi M and Miyaki S 2010 The Mohawk homeobox gene is a critical regulator of tendon differentiation *Proc. Natl Acad. Sci.* **107** 10538–42

Jaiswal D, Cowley N, Bian Z, Zheng G, Claffey K P and Hoshino K 2017 Stiffness analysis of 3D spheroids using microtweezers *PLoS One* **12** e0188346

Jaiswal D, James R, Shelke N B, Harmon M D, Brown J L, Hussain F and Kumbar S G 2015 Gelatin nanofiber matrices derived from Schiff base derivative for tissue engineering applications *J. Biomed. Nanotechnol.* **11** 2067–80

Knobloch K, Grasemann R, Spies M and Vogt P M 2007 Intermittent KoldBlue cryotherapy of 3 × 10 min changes mid-portion Achilles tendon microcirculation *Br. J. Sports Med.* **41** e4

Kraus A, Woon C, Raghavan S, Megerle K, Pham H and Chang J 2013 Co-culture of human adipose-derived stem cells with tenocytes increases proliferation and induces differentiation into a tenogenic lineage *Plast. Reconstr. Surg.* **132** 754e–66e

Kreis T and Vale R 1999 *Guidebook to the Extracellular Matrix, Anchor, and Adhesion Proteins* (New York: Sambrook and Tooze Publications at Oxford University Press)

Kryger G S, Chong A K, Costa M, Pham H, Bates S J and Chang J 2007 A comparison of tenocytes and mesenchymal stem cells for use in flexor tendon tissue engineering *J. Hand Surg. Am.* **32** 597–605

Lavagnino M, Arnoczky S P and Gardner K 2011 *In situ* deflection of tendon cell-cilia in response to tensile loading: an *in vitro* study *J. Orthop. Res.* **29** 925–30

Lavagnino M, Wall M E, Little D, Banes A J, Guilak F and Arnoczky S P 2015 Tendon mechanobiology: current knowledge and future research opportunities *J. Orthop. Res.* **33** 813–22

Leckband D E, le Duc Q, Wang N and de Rooij J 2011 Mechanotransduction at cadherin-mediated adhesions *Curr. Opin. Cell Biol.* **23** 523–30

Lee J, Guarino V, Gloria A, Ambrosio L, Tae G, Kim Y H, Jung Y, Kim S-H and Kim S H 2010 Regeneration of Achilles' tendon: the role of dynamic stimulation for enhanced cell proliferation and mechanical properties *J. Biomater. Sci. Polym. Ed.* **21** 1173–90

Lee K I, Lee J S, Kim J G, Kang K T, Jang J W, Shim Y B and Moon S H 2013 Mechanical properties of decellularized tendon cultured by cyclic straining bioreactor *J. Biomed. Mater. Res.* A **101** 3152–8

Lephart S M, Pincivero D M, Giraido J L and Fu F H 1997 The role of proprioception in the management and rehabilitation of athletic injuries *Am. J. Sports Med.* **25** 130–7

Liu C-F, Aschbacher-Smith L, Barthelery N J, Dyment N, Butler D and Wylie C 2011 What we should know before using tissue engineering techniques to repair injured tendons: a developmental biology perspective *Tissue Eng.* B **17** 165–76

Liu W, Chen B, Deng D, Xu F, Cui L and Cao Y 2006 Repair of tendon defect with dermal fibroblast engineered tendon in a porcine model *Tissue Eng.* **12** 775–8

Luo Q, Song G, Song Y, Xu B, Qin J and Shi Y 2009 Indirect co-culture with tenocytes promotes proliferation and mRNA expression of tendon/ligament related genes in rat bone marrow mesenchymal stem cells *Cytotechnology* **61** 1

Maganaris C N and Narici M V 2005 Mechanical properties of tendons *Tendon Injuries* ed N Maffulli, P Renström and W B Leadbetter (London: Springer) pp 14–21

Maganaris C N and Paul J P 1999 *In vivo* human tendon mechanical properties *J. Physiol.* **521** 307–13

Maganaris C N and Paul J P 2000 Hysteresis measurements in intact human tendon *J. Biomech.* **33** 1723–7

Magnusson S P, Heinemeier K M and Kjaer M 2016 Collagen homeostasis and metabolism *Metabolic Influences on Risk for Tendon Disorders* ed P Ackermann and D Hart (Berlin: Springer) pp 11–25

Manoukian O S, Matta R, Letendre J, Collins P, Mazzocca A D and Kumbar S G 2017 *Biomedical Nanotechnology* (London: Springer) pp 261–78

MATEsystems 2014 Mechano-Active Tissue Engineering www.matesystems.com (accessed July 2019)

Matheson L A, Fairbank N J, Maksym G N, Santerre J P and Labow R S 2006 Characterization of the Flexcell™ Uniflex™ cyclic strain culture system with U937 macrophage-like cells *Biomaterials* **27** 226–33

McNeilly C, Banes A, Benjamin M and Ralphs J 1996 Tendon cells *in vivo* form a three dimensional network of cell processes linked by gap junctions *J. Anat.* **189** 593

Murchison N D, Price B A, Conner D A, Keene D R, Olson E N, Tabin C J and Schweitzer R 2007 Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons *Development* **134** 2697–708

Muschler J L and Horwitz A F 1991 Down-regulation of the chicken alpha 5 beta 1 integrin fibronectin receptor during development *Development* **113** 327–37

Nam H Y, Raghavendran H R B, Pingguan-Murphy B, Abbas A A, Merican A M and Kamarul T 2017 Fate of tenogenic differentiation potential of human bone marrow stromal cells by uniaxial stretching affected by stretch-activated calcium channel agonist gadolinium *PLoS One* **12** e0178117

Nichols A E, Settlage R E, Werre S R and Dahlgren L A 2018 Novel roles for scleraxis in regulating adult tenocyte function *BMC Cell Biol.* **19** 14

O'Brien M 1997 Structure and metabolism of tendons *Scand. J. Med. Sci. Sports* **7** 55–61

O'Brien T D, Reeves N D, Baltzopoulos V, Jones D A and Maganaris C N 2010 Mechanical properties of the patellar tendon in adults and children *J. Biomech.* **43** 1190–5

Orr S B, Chainani A, Hippensteel K J, Kishan A, Gilchrist C, Garrigues N W, Ruch D S, Guilak F and Little D 2015 Aligned multilayered electrospun scaffolds for rotator cuff tendon tissue engineering *Acta Biomater.* **24** 117–26

Ouyang H W, Goh J C, Thambyah A, Teoh S H and Lee E H 2003 Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon *Tissue Eng.* **9** 431–9

Peach M S, James R, Toti U S, Deng M, Morozowich N L, Allcock H R, Laurencin C T and Kumbar S G 2012 Polyphosphazene functionalized polyester fiber matrices for tendon tissue engineering: *in vitro* evaluation with human mesenchymal stem cells *Biomed. Mater.* **7** 045016

Peach M S, Ramos D M, James R, Morozowich N L, Mazzocca A D, Doty S B, Allcock H R, Kumbar S G and Laurencin C T 2017 Engineered stem cell niche matrices for

rotator cuff tendon regenerative engineering *PLoS One* **12** e0174789
Peltonen J, Cronin N J, Stenroth L, Finni T and Avela J 2013 Viscoelastic properties of the Achilles tendon *in vivo* *Springer Plus* **2** 212
Peroglio M, Gaspar D, Zeugolis D I and Alini M 2018 Relevance of bioreactors and whole tissue cultures for the translation of new therapies to humans *J. Orthop. Res.* **36** 10–21
Pioletti D P and Rakotomanana L R 2000 On the independence of time and strain effects in the stress relaxation of ligaments and tendons *J. Biomech.* **33** 1729–32
Popova S N, Rodriguez-Sánchez B, Lidén Å, Betsholtz C, Van Den Bos T and Gullberg D 2004 The mesenchymal α11β1 integrin attenuates PDGF-BB-stimulated chemotaxis of embryonic fibroblasts on collagens *Dev. Biol.* **270** 427–42
Qin T-W, Sun Y-L, Thoreson A R, Steinmann S P, Amadio P C, An K-N and Zhao C 2015 Effect of mechanical stimulation on bone marrow stromal cell–seeded tendon slice constructs: a potential engineered tendon patch for rotator cuff repair *Biomaterials* **51** 43–50
Ralphs J, Waggett A and Benjamin M 2002 Actin stress fibres and cell–cell adhesion molecules in tendons: organisation *in vivo* and response to mechanical loading of tendon cells *in vitro* *Matrix Biol.* **21** 67–74
Ramos D M, Abdulmalik S, Arul M R, Rudraiah S, Laurencin C T, Mazzocca A D and Kumbar S G 2019 Insulin immobilized PCL-cellulose acetate micro-nanostructured fibrous scaffolds for tendon tissue engineering *Polym. Adv. Technol.* **30** 1205–15
Ravichandran A, Liu Y and Teoh S H 2018 Bioreactor design towards generation of relevant engineered tissues: focus on clinical translation *J. Tissue Eng. Regen. Med.* **12** e7–e22
Rawson S, Cartmell S and Wong J 2013 Suture techniques for tendon repair; a comparative review *Muscles Ligaments Tendons J.* **3** 220
Reyes A, Jahr H, van Schie H, Weinans H and Zadpoor A 2014 Prediction of the elastic strain limit of tendons *J. Mech. Behav. Biomed. Mater.* **30** 324–38
Riboh J, Chong A K, Pham H, Longaker M, Jacobs C and Chang J 2008 Optimization of flexor tendon tissue engineering with a cyclic strain bioreactor *J. Hand Surg. Am.* **33** 1388–96
Richardson S H, Starborg T, Lu Y, Humphries S M, Meadows R S and Kadler K E 2007 Tendon development requires regulation of cell condensation and cell shape via cadherin-11-mediated cell-cell junctions *Mol. Cell. Biol.* **27** 6218–28
Riveline D, Zamir E, Balaban N Q, Schwarz U S, Ishizaki T, Narumiya S, Kam Z, Geiger B and Bershadsky A D 2001 Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism *J. Cell Biol.* **153** 1175–86
Robi K, Jakob N, Matevz K and Matjaz V 2013 *Current Issues in Sports and Exercise Medicine* (London: IntechOpen)
Rohatgi R, Milenkovic L and Scott M P 2007 Patched1 regulates hedgehog signaling at the primary cilium *Science* **317** 372–6
Rottner K, Hall A and Small J 1999 Interplay between Rac and Rho in the control of substrate contact dynamics *Curr. Biol.* **9** 640–S1
Saber S, Zhang A Y, Ki S H, Lindsey D P, Smith R L, Riboh J, Pham H and Chang J 2010 Flexor tendon tissue engineering: bioreactor cyclic strain increases construct strength *Tissue Eng.* A **16** 2085–90
Schiele N R, Marturano J E and Kuo C K 2013 Mechanical factors in embryonic tendon development: potential cues for stem cell tenogenesis *Curr. Opin. Biotechnol.* **24** 834–40
Schlegel T F, Hawkins R J, Lewis C W, Motta T and Turner A S 2006 The effects of augmentation with swine small intestine submucosa on tendon healing under tension: histologic and mechanical evaluations in sheep *Am. J. Sports Med.* **34** 275–80
Schneider P R, Buhrmann C, Mobasheri A, Matis U and Shakibaei M 2011 Three-dimensional high-density co-culture with primary tenocytes induces tenogenic differentiation in mesenchymal stem cells *J. Orthop. Res.* **29** 1351–60
Schoenwaelder S M and Burridge K 1999 Bidirectional signaling between the cytoskeleton and integrins *Curr. Opin. Cell Biol.* **11** 274–86
Schweitzer R, Chyung J H, Murtaugh L C, Brent A E, Rosen V, Olson E N, Lassar A and Tabin C J 2001 Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments *Development* **128** 3855–66
Shearn J T, Kinneberg K R, Dyment N A, Galloway M T, Kenter K, Wylie C and Butler D L 2011 Tendon tissue engineering: progress, challenges, and translation to the clinic *J. Musculoskelet. Neuronal Interact.* **11** 163
Stanley R L, Fleck R A, Becker D L, Goodship A E, Ralphs J R and Patterson-Kane J C 2007 Gap junction protein expression and cellularity: comparison of immature and adult equine digital tendons *J. Anat.* **211** 325–34
Stouffer D, Butler D and Hosny D 1985 The relationship between crimp pattern and mechanical response of human patellar tendon-bone units *J. Biomech. Eng.* **107** 158–65
Subramanian A and Schilling T F 2015 Tendon development and musculoskeletal assembly: emerging roles for the extracellular matrix *Development* **142** 4191–204
Synthecon 3D cell culture biodegradable 3D scaffolds https://synthecon.com/pages/home.asp (accessed July 2019)
Szczesny S E, Peloquin J M, Cortes D H, Kadlowec J A, Soslowsky L J and Elliott D M 2012 Biaxial tensile testing and constitutive modeling of human supraspinatus tendon *J. Biomech. Eng.* **134** 021004
TA Instruments Tissue Engineering Instruments www.tainstruments.com (accessed July 2019)
Testa S, Costantini M, Fornetti E, Bernardini S, Trombetta M, Seliktar D, Cannata S, Rainer A and Gargioli C 2017 Combination of biochemical and mechanical cues for tendon tissue engineering *J. Cell. Mol. Med.* **21** 2711–19
Thomopoulos S, Parks W C, Rifkin D B and Derwin K A 2015 Mechanisms of tendon injury and repair *J. Orthop. Res.* **33** 832–9
Thorfinn J, Angelidis I, Gigliello L, Pham H, Lindsey D and Chang J 2012 Bioreactor optimization of tissue engineered rabbit flexor tendons *in vivo J. Hand Surg. (Eur. Vol.)* **37** 109–14
Tiger C-F, Fougerousse F, Grundström G, Velling T and Gullberg D 2001 α11β1 integrin is a receptor for interstitial collagens involved in cell migration and collagen reorganization on mesenchymal nonmuscle cells *Dev. Biol.* **237** 116–29
Vailas A, Tipton C, Laughlin H, Tcheng T and Matthes R 1978 Physical activity and hypophysectomy on the aerobic capacity of ligaments and tendons *J. Appl. Physiol.* **44** 542–6
Vijayavenkataraman S, Shuo Z, Fuh J and Lu W 2017 Design of three-dimensional scaffolds with tunable matrix stiffness for directing stem cell lineage specification: an in silico study *Bioengineering* **4** 66
Waggett A D, Benjamin M and Ralphs J R 2006 Connexin 32 and 43 gap junctions differentially modulate tenocyte response to cyclic mechanical load *Eur. J. Cell Biol.* **85** 1145–54
Wall M and Banes A 2005 Early responses to mechanical load in tendon: role for calcium signaling, gap junctions and intercellular communication *J. Musculoskelet. Neuronal Interact.* **5** 70–84
Wang J H C 2006 Mechanobiology of tendon *J. Biomech.* **39** 1563–82
Wang T, Chen P, Zheng M, Wang A, Lloyd D, Leys T, Zheng Q and Zheng M H 2018a *In vitro* loading models for tendon mechanobiology *J. Orthop. Res.* **36** 566–75
Wang T, Lin Z, Day R E, Gardiner B, Landao-Bassonga E, Rubenson J, Kirk T B, Smith D W, Lloyd D G and Hardisty G 2013 Programmable mechanical stimulation influences tendon homeostasis in a bioreactor system *Biotechnol. Bioeng.* **110** 1495–507

Wang T, Lin Z, Ni M, Thien C, Day R E, Gardiner B, Rubenson J, Kirk T B, Smith D W and Wang A 2015 Cyclic mechanical stimulation rescues achilles tendon from degeneration in a bioreactor system *J. Orthop. Res.* **33** 1888–96

Wang T, Thien C, Wang C, Ni M, Gao J, Wang A, Jiang Q, Tuan R S, Zheng Q and Zheng M H 2018b 3D uniaxial mechanical stimulation induces tenogenic differentiation of tendon-derived stem cells through a PI3K/AKT signaling pathway *FASEB J.* **32** 4804–14

Wang X T, Ker R F and Alexander R M 1995 Fatigue rupture of wallaby tail tendons *J. Exp. Biol.* **198** 847–52

Wang Y, Zhang X, Huang H, Xia Y, Yao Y, Mak A F T, Yung P S H, Chan K-M, Wang L and Zhang C 2017 Osteocalcin expressing cells from tendon sheaths in mice contribute to tendon repair by activating Hedgehog signaling *elife* **6** e30474

Wilmink J, Wilson A and Goodship A 1992 Functional significance of the morphology and micromechanics of collagen fibres in relation to partial rupture of the superficial digital flexor tendon in racehorses *Res. Vet. Sci.* **53** 354–9

Woon C Y, Kraus A, Raghavan S S, Pridgen B C, Megerle K, Pham H and Chang J 2011 Three-dimensional-construct bioreactor conditioning in human tendon tissue engineering *Tissue Eng.* A **17** 2561–72

Wren T A, Lindsey D P, Beaupré G S and Carter D R 2003 Effects of creep and cyclic loading on the mechanical properties and failure of human Achilles tendons *Ann. Biomed. Eng.* **31** 710–17

Wu F, Nerlich M and Docheva D 2017a Tendon injuries: basic science and new repair proposals *EFORT Open Rev.* **2** 332–42

Wu S, Wang Y, Streubel P N and Duan B 2017b Living nanofiber yarn-based woven biotextiles for tendon tissue engineering using cell tri-culture and mechanical stimulation *Acta Biomater.* **62** 102–15

Xu B, Song G and Ju Y 2011 Effect of focal adhesion kinase on the regulation of realignment and tenogenic differentiation of human mesenchymal stem cells by mechanical stretch *Connect. Tissue Res.* **52** 373–9

Xu B, Song G, Ju Y, Li X, Song Y and Watanabe S 2012 RhoA/ROCK, cytoskeletal dynamics, and focal adhesion kinase are required for mechanical stretch-induced tenogenic differentiation of human mesenchymal stem cells *J. Cell. Physiol.* **227** 2722–9

Xu Y, Dong S, Zhou Q, Mo X, Song L, Hou T, Wu J, Li S, Li Y and Li P 2014 The effect of mechanical stimulation on the maturation of TDSCs-poly (L-lactide-co-e-caprolactone) /collagen scaffold constructs for tendon tissue engineering *Biomaterials* **35** 2760–72

Xu Y, Wang Q, Li Y, Gan Y, Li P, Li S, Zhou Y and Zhou Q 2015 Cyclic tensile strain induces tenogenic differentiation of tendon-derived stem cells in bioreactor culture *Biomed. Res. Int.* **2015** 790804

Yamada K M and Geiger B 1997 Molecular interactions in cell adhesion complexes *Curr. Opin. Cell Biol.* **9** 76–85

Yang G, Rothrauff B B, Lin H, Gottardi R, Alexander P G and Tuan R S 2013 Enhancement of tenogenic differentiation of human adipose stem cells by tendon-derived extracellular matrix *Biomaterials* **34** 9295–306

Youngstrom D W and Barrett J G 2016 Engineering tendon: scaffolds, bioreactors, and models of regeneration *Stem Cells Int.* **2016** 3919030

Youngstrom D W, Rajpar I, Kaplan D L and Barrett J G 2015 A bioreactor system for *in vitro* tendon differentiation and tendon tissue engineering *J. Orthop. Res.* **33** 911–18

Zamir E, Katz M, Posen Y, Erez N, Yamada K M, Katz B-Z, Lin S, Lin D C, Bershadsky A and Kam Z 2000 Dynamics and segregation of cell–matrix adhesions in cultured fibroblasts *Nat. Cell Biol.* **2** 191

Zhang J and Wang J H 2013 The effects of mechanical loading on tendons—an *in vivo* and *in vitro* model study *PLoS One* **8** e71740

Zolnoski B, Fernschild E and Jaiswal D 2019 Bioreactor for mechanical stimulation of 3D scaffolds for tendon tissue engineering *45th Ann. Northeast Bioengineering Conf. (NEBEC) (Piscataway, NJ, 20–22 March 2019)*