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CELL BEHAVIOR MANIPULATION (S WILLERTH, SECTION EDITOR)

Using Tools in Mechanobiology to Repair Tendons

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Abstract

Purpose of Review The purpose of this review is to describe the mechanobiological mechanisms of tendon repair as well as outline current and emerging tools in mechanobiology that might be useful for improving tendon healing and regeneration. Over 30 million musculoskeletal injuries are reported in the US per year and nearly 50% involve soft tissue injuries to tendons and ligaments. Yet current therapeutic strategies for treating tendon injuries are not always successful in regenerating and returning function of the healing tendon.

Recent Findings The use of rehabilitative strategies to control the motion and transmission of mechanical loads to repairing tendons following surgical reattachment is beneficial for some, but not all, tendon repairs. Scaffolds that are designed to recapitulate properties of developing tissues show potential to guide the mechanical and biological healing of tendon following rupture. The incorporation of biomaterials to control alignment and reintegration, as well as promote scar-less healing, are also promising. Improving our understanding of damage thresholds for resident cells and how these cells respond to bioelectrical cues may offer promising steps forward in the field of tendon regeneration.

Summary The field of orthopedics continues to advance and improve with the development of regenerative approaches for musculoskeletal injuries, especially for tendon, and deeper exploration in this area will lead to improved clinical outcomes.

Keywords Tendon · Stem cells · Muscle · Scaffolds · Immobilization · Rehabilitation

Introduction

Tendons are highly susceptible to injury and are difficult to treat. Of the 33 million musculoskeletal injuries reported in the USA per year, approximately 50% involve soft tissue injuries, such as tendon injury [1]. A variety of conditions are associated with tendon injuries, from acute tendon tear to tendinopathies to full-width tendon lacerations. Tendonitis, characterized by inflammation or irritation of tendon, is initiated by mechanical overuse and can result in pain, tenderness, tendon damage, and collagen breakdown. Because of its hierarchical structure, tendon inflammation can occur in the tissue surrounding the tendon sheath (paratenon) or inside the tendon (intratenon) [2]. Chronic deterioration of tendon, known as tendinosis, results from chronic overuse and is primarily responsible for decreased strength and flexibility, as well as pain [3]. Tendinosis results from accumulated damage initiated by structural and mechanical overuse and is most often seen in athletic, male runners between 35 and 45 years of age [4]. In patients with chronic Achilles pain, ~70% have tendinosis [5]. The recommended time for return-to-sport is 3–6 months and dependent on rehabilitative strategies, magnitude of tendon injury and pain, and level of sport performance [4]. Achilles tendon rupture and rotator cuff tears are the most common and detrimental tendon injuries [6, 7]. The primary focus of this review is to improve tendon healing following rupture, which is the most common tendon injury that often require surgical repair.

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Increased age and participation in sports are the most common causes of tendon injuries [8]. Sports with abrupt changes in speed and footwork are at risk for Achilles tendon rupture. Although surgical approaches have improved in recent years and can lead to improvements in pain management [9], there remains a low return-to-sport rate following tendon repair. Achilles injuries have the lowest return-to-play as well as the worst post-operative performance compared with other tendon repairs following injury [10]. For example, studies of National Basketball Association players experiencing tendon injuries showed, on average, that return-to-sport rates were upwards of 11 months [11]. Tendon injuries also influence the level of competition that athletes return to following injury. For example, athletes that experience rotator cuff tears do not typically return to the same level as they were prior to their injury [12]. It remains unknown what the exact type or magnitude of loading leads to tendon ruptures, and the physiological history of the tendon also predisposes tendon to damage and injury. In this review, we examine the role that mechanical loading plays in susceptibility for tendon injuries, such as ruptures, and highlight current approaches for improving tendon healing. In addition, we discuss emerging approaches for understanding tissue damage and remodeling from adjacent fields that could be translated to the tendon field in the future.

Mechanical Loading and Overuse Lead to Tendon Injury

Tendons are critical for transmitting contractile forces from muscle to bone in order to move joints as well as provide postural stability. The structure of tendon is a hierarchical cord-like network of aligned collagen fibers that provides a flexible and inextensible connection for load transmission, joint mobility, and joint stability. During growth, tendons are highly adaptable to changes in the magnitude and direction of applied mechanical loads [13, 14]. This adaptive response to applied mechanical load results in tissue-scale adaptations such as increased cross-sectional area, inclusion of compressive-resistant inclusions (fibrocartilage), and tendon length. This can also lead to changes in cellular morphology, density, and behavior, as well as extracellular matrix composition. The developing tendon is highly cellular and actively remodels to meet the applied mechanical demands. During skeletal growth, the tendon lengthens in parallel with bone length and rapidly remodels to accommodate forces from skeletal muscle. However, once the skeleton stops growing, so too does the tendon, and its ability to continuously renew dramatically slows down. This was elegantly illustrated in work by Heinemeier and colleagues with C^{14} bomb-pulse experiments of human tendons [15]. In this study, forensic tendon samples from cadavers were analyzed from humans of varying ages that were alive between 1955 and 1963, when levels of C^{14} were elevated because of nuclear bomb tests. Tendons from human cadavers that were alive during atomic bomb testing were analyzed for radioactive C^{14}, and the levels of C^{14} in tendon were correlated with environmental C^{14} levels. The tendons from humans that were < 17 years of age during this time had low levels of C^{14}, suggesting that the resident tendon cells were able to turnover the C^{14}-labeled tissue at young ages, whereas tendons from older human cadavers had elevated C^{14} levels, indicating that tendon cores did not renew after skeletal growth commenced [15]. Although mature tendons are also capable of adapting and remodeling, this adaptive response is damped because the dense connective tissue of tendon carries much of the mechanical load, shielding the embedded stromal cells, such as tendon fibroblasts, tendon-derived stem cells (TDSCs), and resident inflammatory cells to such loads. The stress-shielding behavior of matrix-dense tendon may influence the longevity of its resident cell populations. In fact, bomb-pulse studies have recently shown that adult tendon does not undergo renewal, which may influence the inherent regenerative capacity of tendon following injury [15].

Changes in the mechanical environment, such as overuse and overloading, are common causes of tendinopathies in mature and aging tendons. Following overuse, the mechanical properties of the tendons deteriorate, as collagen microtears, inflammation, and damage accumulate and are not sufficiently repaired [16, 17]. Adult tendon is unable to regenerate its native structure following injury or damage accumulation, and following injury, the mechanical properties of healed tendon are nearly an order of magnitude lower than the native, healthy tendon [18]. Loading not only alters the structure and function of tendon, it also changes the cellular phenotypes and biological response. Increased mechanical loading leads to increased strain and tissue/microscale damage to the structure and organization of tendon. Tendon overloading leads to reduced microscale collagen alignment as well as nuclear disorganization [19•]. Overuse also results in an increased density of mast cells, which induce a focal inflammatory response and release of prostaglandins and inflammatory cytokines [20]. Overuse leads to increased expression of mechanically induced growth factors as well as proliferation of TDSCs [21]. Understanding how mechanical load plays a role in tendon injury and repair can allow scientists and clinicians to develop rehabilitative approaches in order to heal injured tendons.

There is a need to address tendon healing, including research to understand healing as well as nonsurgical methods to heal tendinopathies. Cells and tissues sense and respond to mechanical and chemical cues from their surrounding extracellular matrix environment, and innovative tools in mechanobiology have emerged as potential therapeutics for treating tendon injuries. This review will highlight current clinical interventions as well as explore potential therapies that could be applied to tendons, including innovative tools that build and mechanically load tissues, guide cellular behavior, and ultimately aim to regenerate the injured tendon.

Current Strategies to Improve Tendon Repair

Tendon heals by forming scar tissue, and many of the current approaches for restoring tendon function following injury aim to intervene in the healing process of tendon [1]. Nonsurgical treatment of tendon injury has varied success rates and depend on the patient and their expectations of the level of functional return that they hope to achieve. Current strategies to improve tendon repair focus on controlling the mechanical environment, extracellular matrix, and cellular behavior of stromal and stem cells (Fig. 1).

Controlled Mechanical Loading Through Immobilization

Immobilization is a common strategy used in most treatment protocols for tendinopathy. However, its efficacy in tendon healing is debated. A framework has been prescribed by the American Shoulder and Elbow Therapists for a rehabilitation strategy of a short-term (2 weeks) strict immobilization period following arthroscopic rotator cuff repair [22]. Following immobilization, controlled loading is often beneficial for promoting healing and reintegration of tendon back to its bony footprint [23]. Yet for chronic rotator cuff tears, there remains no clear advantage or disadvantage for implementing passive mobility as a therapy regime following rotator cuff tendon repair [24]. Early mobility (at ~ 8 weeks post-repair in humans) following immobilization with a cast or boot is typically considered beneficial following Achilles tendon rupture regardless of the tendon underwent surgically repaired [25] as

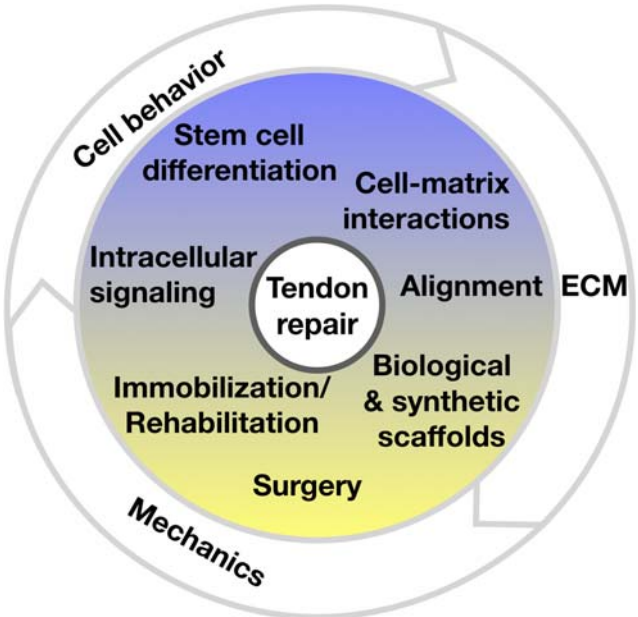

Fig. 1 Tendon repair is mediated by the mechanical environment, native and extrinsic cell behavior, and the structural and material properties of the extracellular matrix (ECM)

it can reduce complication rates and improve return-to-function [26]. The prescribed time of immobilization varies across treatment protocols and the type of tendon injury. In addition, immobilization may only be beneficial until a certain point, after which its prolonged effects can be detrimental. In animal models of tendon rupture, prolonged immobilization can limit return of function and lead to impaired biomechanical properties similar to that of native tendon following rupture [27, 28]. Thus, as a strategy to improve tendon repair, long-term immobilization may not be preferred. The healing process following tendon injury can be delayed with immobilization, as nerve regeneration, blood circulation, and tissue regeneration surrounding the injury site are inhibited [27]. In basic science studies that use external structures or wire framing to immobilize the rat Achilles tendon, the healing tendon repairs with inferior tensile strength, reduced failure load, and decreased stiffness [29, 30]. Immobilization using casting had similar outcomes, as well as increased collagen degradation and decreased collagen mass in the injured tendon/ligament [31]. Additionally, rigid immobilization may lead to atrophy of the surrounding muscle, posing a risk for potential re-rupture [31].

The prescribed implementation and duration of immobilization depend on injury and tendon type. A short bout of immobilization following injury can limit gap formation and improve reintegration between injured tendon stumps [32]. The effects of tendon immobilization on healing also have varying, age-depending effects. For example, following flexor tendon rupture, immobilization and early remobilization of neonatal tendons did not have differential impacts on range of motion, re-rupture, or risk of adhesion, which differs from adult tendons that benefit from early mobilization [33]. Thus, depending on the tendon type, immobilization may be detrimental to the healing process, leading to detrimental changes in the structural integrity of the surrounding muscle and bone.

Surgical Repair and Reattachment of Tendons Post-injury

Surgical treatment of tendon injuries is dependent on the age and health of the patient and the type of tendon tear and involves both surgical approach and post-operative rehabilitation [34]. For young adults, surgical repair remains the main treatment plan given a limited success with nonsurgical intervention without rehabilitation [35]. Surgical approach is largely more successful than a nonsurgical approach alone. Nonsurgical treatment of acute Achilles tendon ruptures, followed by functional rehabilitation, maintained an equivalent rate of re-rupture with surgical treatment [35]. Surgical repair can also reduce the rate of return to work [34]. However, these approaches are not without its limitations and complications. Surgical interventions increase the risk of complications and the risk of infection, nerve injury, and deep

vein thrombosis are significantly increased after operation [36]. Surgical repair procedures, in addition to increased risk of complication, may also not be the logical choice depending on the type of injury, especially not for large and massive tears. For example, success rates, classified by ultrasound or magnetic resonance imaging, of outcomes following rotator cuff repair differ depending on the tear size. Small to medium (1–3 cm) tear repairs typically have high success rates, while the success rates of large tear (3–5 cm) and massive tear (2 or more tendons) repairs are substantially lower [37]. Depending on the size of the tear, variability in repair approach and outcomes can vary. A need exists for post-operative or nonsurgical early intervention [38] and protecting the repaired tendon following repair.

Potential Tools to Guide Mechanobiology During Tendon Repair

The transfer of loads from tissues to cellular and intracellular compartments can dramatically influence how cells respond and adapt to physical cues. Cells convert physical cues into biochemical signals, changes in gene expression, and altered interactions with neighboring cells. In addition, physical deformation of intracellular organelles, such as nuclear compression, can have a significant influence on gene transcription by changing the physical positioning or stretching of genes as well as altering the transport of transcription factors across the nuclear envelope [39–41]. Tissue remodeling in response to applied mechanical loads also relies on plasma membrane integrity and transport. Fibroblasts, which are phenotypically similar to resident tendon cells, transport collagen fibrils across the plasma membrane using nonmyosin II-powered transport [42] and cell surface-directed steps of collagen fibrillogenesis [43, 44].

Stem Cell Populations that Guide Extracellular Matrix Composition and Scar Remodeling

Tendon stem/progenitor cells (TSPCs) are responsible for maintaining tendon cell populations throughout life and replenishing the tissue-resident cell pool following injury. TSPCs have universal stem cell characteristics with capacity to form clonal populations, undergo multipotent differentiation, and self-renew [45]. This stem/progenitor pool is organized by its surrounding extracellular matrix (ECM), and the fate of these cells depends on cues from the ECM niche, such as biglycan and fibromodulin [45]. Recently, unique markers of stem/progenitor cells in tendon that contribute to regeneration have been identified using sophisticated approaches, including single-cell RNA sequencing and lineage tracing approaches [46]. Stem cell-mediated regeneration is dependent on cells that express both tubulin polymerization-promoting protein family member 3 ($Tppp3^+$) and platelet-derived growth factor receptor alpha ($Pdgfra^+$), and cells that are $Tppp3^-$ contribute to injury-associated fibrosis [46]. Previously, cell-surface markers of TSPCs included $Sca1^+$ (marker of stem cells), $Cd90^+$ and $Cd44^+$ (markers of fibroblasts), $Cd18^-$ (marker of leukocyte), $Cd34^-$ (marker of vascular/hematopoietic cells), $Cd106^-$ (marker of endothelial cells), and $Cd133^-$ (marker of perivascular cells) [47, 48]. Historically, TDSCs enhance tendon healing following injury in small animal models, but the role that these cells play in scar formation and remodeling following injury still remains unknown. Conversely, S100 calcium-binding protein a4 ($S100a4^+$) has emerged as a potential marker for a large proportion of tendon resident cells that contribute to scar formation during healing [49, 50]. The discrete populations of cells that are differentially marked by Scx and S100a4 suggest that there likely exists a discrete population of cells that may contribute to either regenerative or scar-mediated healing of tendon following injury [50].

Recapitulating the Mechanobiology of Development for Stem Cell Differentiation

Mechanical load is necessary for proper development of the musculoskeletal system [51–53]. Tendon development is biphasic: muscle must first anchor to tendon and then the attached muscle can apply mechanical force for tendon elongation [54]. Myogenic signals from the developing skeletal muscle also promote tendon development [55] and coordinate the formation of preliminary matrix of fibronectin, laminin, and decorin from tendon progenitor cells [56, 57]. Both *Scleraxis* (Scx) and *Mohawk* (Mkx) promote collagen production, the major component of the tendon ECM, and these transcription factors also promote differentiation of stem-like cells to the tendon progenitor cell fate [58–64]. Tendon ECM is rich in structural proteins that become cross-linked into a lattice to surround fibrils [65] and fibril-associated collagens with interrupted triple helices (FACIT) stabilize the ECM and its interaction with cellular integrins [66]. Proteoglycan bridges composed of decorin, fibromodulin, and biglycan are integral for transmitting and resisting tensile stress and collagen maintenance in tendon [59, 65, 67, 68].

Dynamic changes in ECM stiffness and alignment during tendon development influence the behavior and mode of cell proliferation, recruitment, and migration, the latter of which may be mediated by actomyosin contractility [69]. Tendon elongation depends on the recruitment and migration of mesenchymal progenitor cells via *Scx* [70••]. Differentiation of mesenchymal progenitors is mediated by transforming growth factor (TGF)-β and fibroblast growth factor signaling [71, 72•, 73]. Tendon elongation occurs in parallel with skeletal growth once skeletal muscle is anchored to tendon [13, 74]. Applied mechanical loading from contractile muscle leads to

reorganization of collagen fibrils into parallel and hierarchical fiber structures [65, 67]. Under strain, collagen fibril size and volume fraction increase in developing tendons in accordance with Wolff's Law to generate tendon of higher tissue strength and stiffness [75–77]. Increased size of collagen fibers during modeling requires remodeling of nascent tissues through matrix metalloprotease (MMP) that unwind and break collagen fibrils [78–80]. Mature collagen fibrils harbor functional integrin-binding sites called latency-associated peptides (LAP) structures [81] that also release TGFβ [82]. TGFβ activates Smad2/3 signaling pathways to promote the additional release of TGFβ from the ECM, and this release decreases with increasing magnitudes of mechanical load [83–85]. Larger, highly organized collagen fibrils provide tendon with a capacity to handle increased tensile loads [86]. Collagen remodeling relies on active matrix metalloproteinase (MMP) activity [87, 88], and a balance between collagen matrix production and breakdown is important during the healing process. The dynamic change in matrix stiffness during development can guide stem-like cells towards a fibroblastic cell fate [89]. Collagen synthesis in biological and synthetic ECM, such as scaffolds, can be fine-tuned with localized growth factor signaling, such as platelet-derived growth factor (PDGF) receptor or PDGF-BB [90, 91] and insulin-like growth factor (IGF-1) [90, 92].

Biomimetic Scaffolds for Guided Tendon Repair

The use of scaffolds to bridge tendon defects, guide remodeling, and accelerate healing has been a promising approach for preclinical studies but has demonstrated mixed results for improving patient outcomes when translated to the clinic. In fact, there are few long-term studies that have supported the use of various scaffolds for tendon repair. Nonetheless, scaffolds offer a mechanical advantage over more simple reattachment repair approaches, especially for rotator cuff repair [93–95]. In addition, over 50,000 patches derived from extracellular matrix derivatives (e.g., dermis, amniotic membrane) are used in the clinic each year to repair soft tissue injuries [37]. However, although some biologically (ECM)-derived scaffolds enhance cell attachment and new tissue formation, these benefits come at the cost of mechanical integrity and durability. A balance is needed in the design of biomimetic scaffolds to not only provide mechanical strength and durability but also to promote stem cell differentiation, stromal migration, and limit scarring and immunogenic responses.

The most common sources of naturally derived ECM used for tendon-mimetic scaffolds include small intestine submucosa, amniotic membrane [96], collagen [97], gelatin (denatured collagen) [98], glycosaminoglycans [97], silk [99], hyaluronic acid, and fibrin (described in more detail in a recent review by Freedman and Mooney [100]). Additionally, synthetic ECM has also been used for developing scaffolds for tendon repair, including poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(ethylene glycol) (PEG) [100], poly(ε-caprolactone) (PCL) [101], and combinations of these (e.g., poly(l-lactide-co-ε-caprolactone) (PLCL) [102]), as well as polyacrylamide [100]. The selection of ECM-derived and synthetic materials for use in tendon repair depends on the wide range of mechanical properties both in tension and compression of these materials. Additionally, each material possesses different microscale interactions of cells with ECM, and these properties can be exploited depending on the cell type, density of ECM and cells, and processing used for manufacturing the scaffolds. Rigorous in vitro and preclinical testing of the ECM scaffolds are essential for each iteration of scaffold design.

Cell adhesion, migration, and proliferation are critical for regenerating and revitalizing tissue replacements, and these processes are mediated by cell-cell and cell-matrix interactions. These cellular behaviors are also dependent on the mechanical and biological properties of two- and three-dimensional substrates upon which the cells reside. Cells sense mechanical loads from their surrounding ECM via integrins [83, 84]. Two- and three-dimensional microenvironments, both from synthetic and biologically derived sources, have been used to identify and exploit the cell-cell and cell-matrix interactions. For example, integrin binding sites (e.g., RGD) are commonly used in synthetic materials, such as hydrogels, to promote cell adhesion and invasion in otherwise biologically inert substrates [103]. Cell morphology and differentiation depend on these binding sites, as well as matrix compliance [103, 104]. Factors that should be considered when using stem cells in conjunction with biomimetic scaffolds include stem cell priming (i.e., potential benefits and costs of pre-implantation differentiation towards a tenogenic cell fate) and temporal exposure to binding sites that promote proliferation, differentiation, and ECM production.

The development of custom-built bioreactors has helped to define how various parameters of dynamic stretch (e.g., amplitude, frequency, and duration) influence cellular behaviors and phenotypes of tendon fibroblasts and stem cells in vitro [105–108]. Various approaches have been used to build tendon mimics, including directed self-assembly of dermal fibroblasts [106] or primary tendon fibroblasts [109] or contraction of fibrin gels [110].

Bioelectricity

Aside from clinical diagnosis of tendinosis, tendon damage is not well described. However, the ability of tendon-resident cells to sense and respond to mechanical forces, including the cell-scale response to tendon overuse, is a potential target for abrogating the accumulation of damage and improving healing outcomes. A major process of mechanobiology across all tissues, including tendon, relies on membrane permeability

to ions and early intracellular communication [111]. Typically, neurons and myocytes are considered "activatable" cells as they respond to suprathreshold changes in the transport of ions across the membrane via action potentials. However, other cells types, including those found in musculoskeletal tissues, also respond to changes in membrane potential by changes in gene transcription, receptor/protein phosphorylation, and changes in mitochondrial function. Ion transport across the plasma membrane is controlled in part via plasma membrane integrity and has been identified in vivo as an important process for bone cell (e.g., osteocyte) mechanosensation [112••]. Plasma membrane disruptions can regulate expression of early response proteins, such as c-fos, in various cell types, supporting the "damage sensor" hypothesis that can explain how cells initiate adaptative responses to injurious but not lethal mechanical stress [112••, 113]. This hypothesis poses that transient plasma membrane stretch that induces small breaks or tears in the plasma membrane can induce a mechanosensitive cascade of cell signaling if the number or magnitude of tears is large enough. While some plasma disruptions may be beneficial for driving adaptation in response to mechanical load, a threshold likely exists that, when exceeded, results in cell death if the plasma membrane disruption is not repaired. This damage threshold reduces in disease and in aging, which is postulated to increase risk of unrecoverable injury in diseased or aged tissues, such as muscle and bone. Potential therapeutics for maintaining or repairing plasma membrane integrity include antioxidant treatment (e.g., vitamin E [114] or vitamin C [112••]), fetuin A treatment [115], or surfactant poloxamers (such as P-188) [116, 117], and these treatments show promise in maintaining cell viability for muscle, bone, or cartilage following tissue- or cell-scale damage. Although the damage sensing mechanisms in osteocytes and skeletal myocytes have been translated and validated in vivo, it has yet to be demonstrated as a model of mechanobiology in tendon.

In addition to membrane integrity, voltage-gated transport of ions across the membrane (e.g., transient receptor potential cation channel subfamily V member 4, TRPV4; voltage-operated calcium channel, VOCC) may also be controlled via mechanical stress and/or extracellular stiffness [118, 119]. In nonmusculoskeletal cells (e.g., cardiomyocytes), fibroblast growth factor homologs modulate the trafficking of calcium and sodium ion channels to the plasma membrane, which suggests a coordinated role maintaining homeostasis in activatable (and perhaps previously considered "nonactivatable") cells [120].

Conclusions

The progress towards improving tendon healing with translatable therapeutics is promising, and control of cellular and extracellular processes may provide useful and effective treatments for improving repair outcomes. Engineered materials from natural or synthetic sources may be useful for guiding cellular remodeling and improving biomechanical reintegration of ruptured tendon. The incorporation of biological cues that mimic development may be useful for guiding differentiation of stem cells towards a tenogenic fate and accelerate tendon healing. The control of biological processes (e.g., bioelectricity) could potentially be leveraged to encourage tendon regeneration driven by tendon-resident stromal cells. The field of orthopedics continues to advance and improve with the development of regenerative approaches for musculoskeletal injuries, especially for tendon, and deeper exploration in this area will lead to improved clinical outcomes.

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Conflict of Interest The authors declare that they have no conflict of interest.

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