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Orthopedic Interface Repair Strategies Based on Native Structural and Mechanical Features of the Multiscale Enthesis

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Abstract

The enthesis is an organ that connects a soft, aligned tissue (tendon/ligament) to a hard, amorphous tissue (bone) via a fibrocartilage interface. Mechanically, the enthesis sustains a dynamic loading environment that includes tensile, compressive, and shear forces. The structural components of the enthesis act to minimize stress concentrations and control stretch at the interface. Current surgical repair of the enthesis, such as in rotator cuff repair and anterior cruciate ligament reconstruction, aim to bridge the gap between the injured ends via reattachment of soft-to-hard tissues or graft replacement. In this review, we discuss the multiscale, morphological, and mechanical characteristics of the fibrocartilage attachment. Additionally, we review historical and recent clinical approaches to treating enthesis injury. Lastly, we explore new technological advancements in tissue-engineered biomaterials that have shown promise in preclinical studies.

Graphical Abstract

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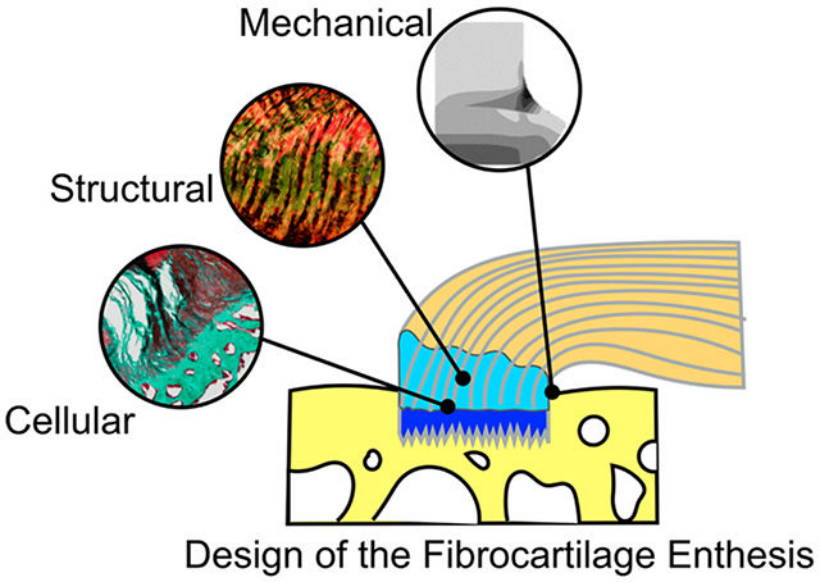

Keywords

enthesis; tendon; ligament; meniscus; bone; mechanics

INTRODUCTION

The burden of musculoskeletal disorders on the economy is significant, resulting in lost wages, time away from work, and an ever-increasing duration of pain and disability.[1] In 2014, 32% of lost work days were related to musculoskeletal injuries.[2] Musculoskeletal injuries involving tendon or ligament rupture often require surgical repair for their functional reattachment back to bone.[3–9] In such cases, the attachment of the connective tissue, known as the enthesis, rarely regenerates after surgical repair[10–12] and can result in a high probability of failure.[13–15] Unfortunately, some clinical cases of enthesis injury, such as rotator cuff tears and anterior cruciate ligament (ACL) rupture, are unresolved due to failure of the tendon/ligament to sufficiently integrate into the bone. The clear clinical problem associated with poor enthesis healing following injury requires understanding of the function, structure, and healing potential of the native attachment. Recapitulating the intact enthesis following injury is the “holy grail” in tendon and ligament reconstruction. Therefore, the development of strategies that guide the reattachment and bridging of soft tissue to bone, resulting in regeneration and return of function and strength of the attachment, are of high priority in the field of orthopedics.

Enthesis injuries can occur at practically any site of tendon/ligament attachment to bone, with the most common sites located at the rotator cuff of the shoulder[16,17] and the cruciate ligaments of the knee.[18,19] Upon surgical repair, the multiscale enthesis structure is difficult to regenerate without guided intervention, leaving the repaired tendon-bone attachment susceptible to failure at rates as high as 94%.[13,14] Re-establishing a robust enthesis is important not only for the return-to-function for many athletes but also for daily function

and independence for individuals of all ages.[20–22] Unfortunately, following repair, pull-out or rupture of the graft-bone interface is the driving cause of failure. For example, nearly 25% of all ACL reconstructions fail due to laxity or failure at the graft–bone interface.[23] Failure of the repaired ACL to integrate into bone can result in knee instability[24,25] that has implications for the early onset of knee osteoarthritis (OA).[23,26] Integration at the graft–bone interface is a problem for other musculoskeletal tissue analogues as well, such as the meniscus. Thus, successful regeneration of the native and strong enthesis is imperative for maintaining long-term joint health. Regenerating a functional and strong interface between graft and bone also presents critical design criteria for developing new biomaterials to treat tendon and ligament injuries that require engrafted replacement of the fibrous tissues. To accomplish congruent, integrated, and functional interfaces, we must understand the multiscale biological, mechanical, and structural incorporation of native tendon/ligament to bone.

The goal of this review is to highlight our current understanding of the multiscale morphology, mechanics, and pathology of the tendon/ligament enthesis, discuss current clinical strategies and obstacles of enthesis repair, and propose new and future directions for incorporating biomaterials in enthesis regeneration.

MORPHOLOGY AND MECHANICS OF THE MULTISCALE ENTHESIS

The enthesis is an organ that connects a soft, aligned tissue (tendon/ligament) to a hard, amorphous tissue (bone) (Figure 1). The morphology of the attachment is generally consistent across fibrocartilage entheses, as shown for ligament (Figure 1A), meniscus (Figure 1B), and tendon (Figure 1C) attachments. In this review, we will use “ligament” and “tendon” interchangeably in reference to enthesis morphology and mechanics. Additionally, we lead with the assumption that the ligament, meniscus, and tendon fibrocartilage enthesis are generally comparable in morphology and mechanical behavior. However, it should be noted that some variations in morphology and biochemistry exist among tendon, meniscus, and ligament as well as, presumably, their associated entheses. However, few have directly compared cross-anatomical entheses associated with ligament, meniscus, and tendon, and direct comparisons of morphological, biochemical, and mechanical properties of the ligament, meniscus, and tendon are not common in the literature. This may be a result in intraconnective tissue heterogeneity (i.e., tendon patterning is dependent on its anatomical and mechanical requirements). We do know that, in general, patellar and Achilles tendons have less DNA and glycosaminoglycan content, but have more collagen (type I), compared to several knee ligaments (e.g., ACL, posterior cruciate ligament, and medial collateral ligament).[27] Historical work has shown that the patellar tendon is stronger and more stiff compared to the ACL, posterior cruciate ligament, and lateral collateral ligament.[28] The differing characteristics noted previously between tendon and ligament are presumed to be related to (1) differences in the rate of maturation between tendon/ligament (e.g., proximal attachments may mature prior to distal attachments), (2) differences in metabolic/functional requirements (e.g., stabilizing ligaments versus large force-generation tendons), and (3) varying anatomical location/proximity to joints.[27] Nonetheless, variability in tissue structure across tendons, let alone among tendon, meniscus, and ligament, exists; therefore, we aim to

describe the fibrocartilage enthesis as it pertains to the connective tissue structures generally.[27]

Variation in the size, alignment, and footprint of the enthesis depends on the loading requirements of the attachment, as explained later in this review. Between the tendon and bone exists the transitional tissue known as the fibrocartilage, which maintains two distinct regions; the calcified fibrocartilage (CF, Figure 2, dark blue region) and the uncalcified fibrocartilage (UF, Figure 2, light blue region). The fibrocartilage forms during skeletal maturation, and the distinct regions are separated by a mineralized tidemark. The enthesis footprint typically forms on a bone eminence, which is defined as a projection, ridge, and tuberosity on the surface of bone that provides bone its topographical shape. The eminence is derived during embryonic development and forms modularly from cells that are descendants of a scleraxis+ and sox9+ cell population.[29,30] The modular formation of the eminence has been suggested to follow a “segregation” model, which describes the initial establishment and subsequent separation of the common enthesis progenitor pool during embryonic growth.[31] The segregation model does not require coordinated signaling for navigation of the tendon to the attachment site; rather, the tendon and enthesis form together and *in situ*. Currently, it is theorized that the pluripotency of the enthesis progenitors allows for the intricate assembly of its unique gradient cellular and extracellular morphology during development rather than coordinated, guided migration of tendon to the eminence.[29,31–33]

The enthesis morphology is, in general, true for all fibrocartilage entheses, regardless of the soft-tissue derivative (i.e., tendon, ligament, or meniscus; Figure 1). The extracellular matrix of the UF includes a multitude of collagen types, including type I, II, III, IX, and X,[12,34–36] as well as elastin fibers, aggrecan, glycosaminoglycans (GAGs), and proteoglycans (PGs).[34] Alignment of the collagen fibrils from the soft tissue to bone (Figure 1D) has been shown to be dependent on the anatomical location,[37] which is presumed to be a function of the loading requirements of the soft tissue. The cellular makeup of the enthesis is unique compared to the fibroblasts of the tendon and osteocytes/osteoblasts of the subchondral bone.[38] The cell shape and organization spatially changes from tendon/ligament to bone in the mature fibrocartilage attachment. Aligned spindle cells reside in the tendon, with a mixture of spindle and round cells (or fibrochondrocytes) in the fibrocartilage and osteoblasts/osteocytes in the SB. During embryonic development, the enthesis cells modularly form the attachment[29,30] and, in mice, have been recently shown to maintain residence throughout growth.[39] Resident enthesis cells display a prehypertrophic, fibrochondrocyte phenotype akin to an arrested growth plate.[39] The size and extent of the fibrocartilage and its degree of mineralization are dependent on the mechanical loading requirements of the attachment (Figure 2B and C).[31,40–42]

Mechanically, the enthesis sustains a dynamic loading environment that includes tensile, compressive, and shear forces (Figure 2B and C). This is accomplished through several structural and compositional elements, which act to minimize stress concentrations and control stretch at the interface. The complex, 3-dimensional fibrocartilage enthesis forms in response to the loading environment during development.[10,12,31,46,47] Upon active loading from contracting skeletal muscle (tendon) or passively through joint motion (ligament), tensile forces from the tendon/ligament are transmitted along aligned collagen fibrils into the

fibrocartilage and then bone (Figure 2B and C). The collagen fiber alignment splays outwardly near the interface and has a profound impact on the location of both maximum principle strain and maximum principle stress.[47,48] The presence of GAGs within the fibrocartilage plays a role in resisting compression of the enthesis due to Poisson's effect;[49,50] interwoven collagen fibers and the accumulation of GAGs maintain an elevated swelling pressure by binding to and locking in water in the fibrocartilage and thus resisting compression. These structural features support the "stretching brake" theory, posited by Knese and Biermann,[51] who described one functional role of the enthesis is to limit the narrowing of the interface under tension. However, tensile loads are not the only loads applied to the enthesis; fibrocartilage attachments develop at anatomical sites where tendons/ligaments function in multiaxis loading. In other words, the footprint of the fibrocartilage attachment is larger than the cross-section of the tendon/ligament itself, which aids in the redirection of forces away from the interface and distributes loads over a larger area (Figure 2).[52] Macroscopically, fibrocartilage formation also experiences compression and shear at the articular surface, which occurs when the tendon wraps around bone rather than inserting directly into bone.[46] The splayed fibrocartilage footprint also prevents the tendon from deforming around a sharp corner at the mineralized interface, acting as a grommet between bone and soft tissue.[10] At the microscale, the tendon also experiences interfibrillar shear from fibril sliding due to load-sharing between fibrils.[53]

The fibrocartilage enthesis also establishes interdigitations at the CF/SB interface (Figure 1B and Figure 2).[37,54] The frequency, size, and distribution of interdigitations at the ligament/bone interface are likely regulated by the mechanical loading environment.[55] Recently, the interdigitation of the tendon–bone attachment has been demonstrated computationally to act as a toughening mechanism between the CF and SB.[39] Deep-rooted interdigitations of ligamentous fibers (e.g., from the tibial ACL anteromedial bundle) derive their depth because of their need to resist multidirectional tensile forces.[56,57] In the diseased state, such as OA, the tidemark at the meniscal UF/CF is disrupted, and interdigitation-like patterning forms in addition to soft-tissue calcium deposits, osteophyte formation, microcracks, fissuring, and duplication of the tidemark.[54] Whether disruption of the interface is a result of or a driving factor in disease progression has yet to be determined.[58]

PATHOLOGICAL FACTORS THAT MODULATE ENTHESIS REGENERATION

Age-related changes can result in permanent changes to the structure and function of the enthesis.[59] Disruption in the congruency of collagen fiber interdigitation,[54] FC mineralization,[54] and/or GAG and proteoglycan distribution[58] across the enthesis are associated with enthesis disorders; however, it is unclear if these changes initiate or are the result of enthesis degeneration. Regardless, enthesis degeneration is prevalent in chronic disorders, such as OA[54] and rotator cuff disease.[60,61] Following injury, the residual stress in tendons and ligaments causes the soft tissues to retract from their bony insertions.[62] Clinically, acute tears of the tendon/ligament have a higher capacity for healing compared to chronic, prolonged detached soft tissues.[63–66] The inflammatory process involved in healing following acute injury has been studied in patients as well as using animal models. Acute injuries, such as following rotator cuff tears, follow the process of natural wound healing. Almost immediately following injury, vascularization and inflammation of the injured

interface can occur.[67–69] Chemotactic factors, such as vascular endothelial growth factor (VEGF), transforming growth factor-b (TGF-β), and platelet derived growth factor (PDGF), are secreted locally and encourage recruitment of fibroblasts to the injury site.[70] Neutrophils, macrophages, and osteoclasts are then recruited to the injury site for remodeling of the damaged tendon, fibrocartilage, and bone.[67,68,70–72] During repair, fibroblasts proliferate and produce ECM proteins including GAGs and collagen type III. Functionality of the tissue is typically restored within months during the last stage of healing, known as the remodeling phase, where deposited collagen type III is converted to highly organized collagen type I. However, the remodeling of enthesis following injury insufficiently recapitulates the native enthesis, and the healed structure is often structurally disorganized and mechanically inferior.[1–3,31,48,63,73–75] Further, the morphology and organization of enthesis cells do not return to normal after injury.[76] Additionally, chronic/large enthesis injuries, such as that observed with massive rotator cuff tears, have a high likelihood of failure to heal following repair.[6,14] It is currently unknown what role the inflammatory process plays in these different healing scenarios. Along with potential variations in the inflammatory response, investigations have elucidated the potential role of mechanical cues in the healing response. Altered mechanical loading from the muscle or joint may differentially regulate the healing response following acute versus chronic tendon/ligament injury.[63,64,68,77,78] While adaptations in bone and muscle structure are evident following chronic tendon-bone injury in several clinical and preclinical studies; the functional outcomes associated with these factors are nuanced.[14,63,64,68,73,74,77,79,80] Additionally, associations with muscle and bone loss can have substantial implications in the health, maintenance, and healing of the enthesis, but these relationships have not yet been clearly defined.

CLINICAL STRATEGIES FOR REPAIRING THE INJURED ENTHESIS

Improvements in repair outcomes have recently relied on leveraging the technological advancements from tissue-engineered biomaterials. For example, mobilization of the tendon to the native bony footprint is difficult due to retraction forces and remodeling of tissue.[74,81,82] Scaffolds that bridge the gap between the tendon stump and bone may provide reinforcement during healing.[83,84] These scaffolds must be strong enough to withstand the physiological and pathophysiological loading of a normal joint. Many preclinical devices for repairing musculoskeletal interfaces, such as interpositional grafting[85] and rotator cuff augmentation,[86] have failed due to inadequate mechanical and structural properties of graft material (e.g., autograft, allograft, xenograft, or tissue engineered scaffold), which ultimately influence graft failure.[87] Unfortunately, there have been few victories in the clinic with utilization of scaffolds for enthesis repair in human patients. Additionally, extracellular matrix scaffolds used clinically have had a limited number of follow-up studies in human patients.[83] The use of cross-linking to stiffen biological scaffolds has been shown to elicit an immune response upon implantation, and several xenogenic patches have led to immunogenic responses.[88,89] Nonetheless, some devices used for patch augmentation and restoration of the rotator cuff tendon-bone attachment, such as GraftJacket,[90,91] have shown some promising results. The GraftJacket is a freeze-dried, human-derived, non-cross-linked, and decellularized dermal allograft that has been implemented clinically in small cohorts of

patients.[90,91] In human clinical trials, full matrix incorporation of the scaffold into the native tissue has been observed via magnetic resonance imaging.[92] Additionally, following rotator cuff repair, patients with GraftJacket augmentation demonstrated improvements in external rotation strength compared to preoperative metrics.[92] However, cadaveric[93] and laboratory[94] studies have demonstrated several potential failure mechanisms related to the GraftJacket, such as suture pull-through and breakage. These data suggest that a combination of improved scaffold properties (structural and mechanical) and suture techniques is important for the success of scaffold-based repair of the rotator cuff and other tendons/ligaments entheses. While these scaffolds act as a patch that physically connects the tendon to bone, their use has been primarily limited to patients with massive, otherwise irreparable tears.[90,91] There exist several issues with the health of the rotator cuff in this distinct patient population, including fat atrophy, weakness of the cuff muscles, and localized subchondral bone loss at the attachment footprint. The verdict is still out as to whether or not patches and grafts, which behave as bridges between the tendon and bone, are capable of restoring the mechanical function of the interface as well as allowing for the patient to regain rotator cuff muscle and bone health. If the functional reattachment of the tendon to bone is enough to overcome the chronic, degenerative changes that occur in rotator cuff disease, then devices like the GraftJacket could revolutionize the way surgeons treat patients with otherwise irreparable tears.

Knee ligament injuries, such as ACL tears, are common sources of musculoskeletal pain.[95,96] ACL tears do not heal, leaving a gap between the injured ends,[97] and surgical reconstruction is the current standard of care.[98–103] Suturing the ends leads to failure rates as high as 17%,[104] while reconstruction using allograft and xenograft has been shown to be limited by immune rejection.[96,105–109] Trials using allografts, such as cadaveric patellar and hamstring tendons, have shown that failure rates following ACL reconstruction increase from 6 to 20%, compared to autografts, in adolescents.[110] Surgical ACL reconstruction using autografts is the current standard of care.[98,99,103] However, autograft ACL reconstruction surgery has been shown to be limited by donor-site morbidity, i.e., complications associated with the graft location.[111] Donor-site morbidity may contribute to increased anterior knee pain and risk of repair failure after autograft reconstruction.[103,110] Several factors have improved the outcomes of ACL reconstruction using autografts, including choice of autograft retrieval location and size, location and size of the bone tunnel, proper graft tension, anatomic graft fixation, and graft fixation strength.[98] Clinical trials using patellar autografts have demonstrated reduced failure compared to hamstring tendon autografts;[99,106] however, patellar autografts resulted in increased rate of anterior knee pain.[99] Additionally, graft failure can occur due to mechanical factors, such as trauma-induced overloading, fatigue of the graft over time, and improper surgical technique.[96,98,112] Potential biological modes of failure include effusion, infection, and bone tunnel widening, primarily at the site of graft-to-bone integration, producing mechanically weaker interfaces that can ultimately fail.[106]

Manufactured scaffolds to replace the ligament[74,113,114] or to bridge the gap between the injured ends[95,97,115] have several potential advantages, including obfuscating disease transmission, design control, sterilization, and biocompatibility.[116] However, few have demonstrated success in clinical trials.[117] While manufactured scaffolds for ligament

replacement are numerous, the long-term disadvantages include fatigue of the material, indicating inadequate material properties for the mechanical loads found *in vivo* and decreased tissue ingrowth. Additionally, there are limited clinical trials using tissue-engineered ligaments. Approved devices, such as Intergraft (carbon fiber),[118] Gore-Tex ACL (polytetrafluoroethylene),[119] Stryker Dacron Ligament (polyethylene terephthalate),[120] Leeds-Keio Artificial Ligament (polyethylene terephthalate),[121] and Kennedy Ligament Augmentation Device (polypropylene),[122] have reported negative results.[114] Intergraft resulted in tunnel widening, synovitis, and high rupture rate.[118] Patients with Gore-Tex and Kennedy ACL reconstructions presented with joint instability and a high rate of effusion and inflammation, and wear particles were found within the synovial fluid.[122] Reconstruction with the Leeds-Keio Artificial Ligament, along with Stryker Dacron Ligament and Kennedy ligaments, resulted in excessive laxity and unacceptable rates of failure ranging from 19% to 80% at long-term follow-ups.[118,119,121,122] These complications led to the removal of all of these devices from the market. The Ligament Augmentation and Reconstruction System (LARS)[118,123] remains on the market and is showing promising results, but it is not available in the United States and lacks long-term follow-up. Current LARS outcomes demonstrate reduced incidence of infection and decreased failure rate compared to that of the previous synthetic grafts.[123] Overall, mechanical and biological failure are the main limitations for synthetic grafts; thus, future grafts should have improved mechanical and biological properties more suited for the biomechanical environment within the injured knee.

Preclinical studies investigating tissue-engineered scaffolds suggest the potential benefit over traditional autograft reconstruction.[95,113,115] Primarily, tissue-engineered scaffolds eliminate the risk of donor-site morbidity.[111] These devices can be industrially manufactured to mimic the native structural and mechanical features of the enthesis and are processed for sterility and biocompatibility. Additionally, combinatorial approaches in tissue-engineered scaffold design can incorporate biological or chemical factors that encourage cellular infiltration, mineralization, and matrix deposition. For example, silk-collagen,[113] silk-tricalcium phosphate-polyether ketone (silk-TCP),[124] and poly-(lactic-co-glycolic acid) (PLGA) triphasic scaffolds[125] have shown decent integration of scaffold-to-bone and may be potential alternatives to autograft reconstruction. Silk-collagen scaffolds induced a similar response to autograft reconstruction with abundant fibroblast-like cell proliferation and positive Tenascin-C staining, and improved trabecular bone growth in the scaffold group.[113] Similarly, silk-TCP scaffolds created a transition of ligament-to-bone similar to native ACL bony insertions, with transitional zones of silk fibers, fibrous tissue, fibrocartilage, TCP, and bone.[124] The PLGA triphasic scaffold maintained distinct cellular regions and phase-specific matrix deposition akin to the native ACL enthesis.[125] Another alternative is bridge-enhanced ACL repair (BEAR), which was shown to have the same mechanical properties as autograft reconstruction and reduced post-traumatic OA in pigs.[115] Future clinical trials with long-term follow-ups are necessary to validate these tissue-engineered scaffolds as alternatives to the current standard of ACL reconstruction.[4]

EMERGING STRATEGIES FOR ENHANCED ENTHESIS HEALING

Designing for success in enthesis augmentation is nuanced, with the mechanical environment an obvious factor to consider.[126] Failures in the clinical and preclinical stages

emphasize the need to develop new strategies to improve outcomes. Current approaches in tissue engineering have shown promising results to improve tendon-to-bone repair compared to the current, standard clinical treatments.[127,128]

The complex structure and function of the enthesis make it difficult to design appropriate devices and reliable strategies for augmentation and repair. Recent tissue-engineering approaches have attempted to recapitulate the local structural environment by capitalizing on the known alignment of collagen fibers at the interface. For example, scaffolds using biodegradable materials, such as silk[129] and collagen,[130] have been manufactured using electrospinning techniques, which result in spatially aligned, thin nanofiber sheets. Electrospun polymers have also been used to generate aligned fiber scaffolds showing potential in preclinical augmentation of the rotator cuff tendon–bone attachment and other musculoskeletal interfaces.[131–150] Several advantages exist for using electrospinning techniques for interfacial scaffolds. The choice of material, pore space and connectivity, density, fibril alignment, mechanical properties, surface area, and functionalization can be controlled during or after electrospinning.[145,151–154] Recent work has shown that the micromechanical properties of single electrospun PLGA fibers can be modified using crystalline coatings of hydroxyapatite.[140] In addition, crimping of electrospun polylactic acid (PLA) nanofibers can be controlled using chemical modifications,[155] airflow/heat,[148,156] magnetic/electric fields[157,158] or self-assembly.[159] Aligned, electrospun fibers with gradations of mineral content[160–162] also hold promise for recapitulating the microscale and nanoscale structure of the enthesis. Such scaffolds can provide a platform for fine control and patterning of cellular differentiation into osteoblasts[163] and potentially chondrocytes and tenocytes as well.[164] Stem cell differentiation can also be controlled by varying the alignment and diameter of electrospun PLGA fibers.[144] The use of electrospinning techniques to create biomaterials that mimic the native structural and mechanical features of the multiscale enthesis holds promise for micropatterning and spatial deposition of biomolecules and scaffold materials to help guide regeneration of the microscale enthesis.

Additive manufacturing, also known as 3-dimensional printing, has become popular for personalized fabrication given its low cost, ease of use, and versatility.[165–167] The use of additive manufacturing with hydrogel-based bioinks[165] and/or selective laser/electron beam melting[168] can aid in design specificity from computer-aided or image-based design.[166,167] Additive manufacturing for medical implantation may hold promise for enthesis prostheses. Assembly of an artificial interface using 3-dimensional printing techniques that allows for controlled deposition of mineral and collagen, with spatially dependent mechanical reinforcement, could potentially revolutionize the current design of graft-bone replacements. Recently, printable hydrogels have been used for the manufacturing of organ-scale constructs of mechanically reinforced templates that promote bone formation, such as vertebral bodies.[165] Additionally, the use of printing techniques to guide bone accrual at the bone/graft interface has shown promise in preclinical models of ACL replacement.[166]

Examining the structural and functional elements of the enthesis reveals how nature overcomes the engineering feat of joining dissimilar materials. Engineering tools and principles have identified key features that contribute to this robust union. The clinical challenge now remains to reassemble these pieces and integrate them in the same fashion as

they develop. The ongoing advancements in understanding the native enthesis continue to guide new and exciting approaches to improve repair strategies and long-term outcomes. Approaches to fine-tune the design of engineered materials will guide the cellular, mechanical, and structural regeneration of the fibrocartilage enthesis (Figure 3). Repairing the damaged enthesis using scaffolds that aid in spatially patterned cell differentiation (e.g., gradient in cell shape from tenocytes to fibrochondrocytes to osteoblasts) can enhance the stem cell differentiation from native or exogenously delivered cells. In order to recapitulate the mechanical environment, we can improve the design of scaffolds to address physiological design criteria. Potential directions include designing scaffolds with gradients in stiffness and mineral between soft tissue and bone, wrap-aligned fibers, spatial deposition of charged macromolecules for localized water storage, and/or fibril cross-linking (Figure 3). These design criteria must meet the needs of the *in vivo* loading environment, such that the scaffold can maintain multiaxis loading and act as a “stretching brake” (Figure 3). Although not exhaustive, such recommendations are currently being pursued in preclinical models and hold potential for bench-to-bedside therapies in the future.

CONCLUSION

The native structural features of the enthesis play a crucial role in the mitigation of stress concentrations at the interface between tendon and bone. After injury, the tendon/ligament retracts from bone due to tensile forces and then undergoes typical wound healing, which produces a mechanically weaker and structurally disorganized tissue. Current clinical techniques used to repair the interface, such as bridging and graft reconstruction, can fail due to inadequate, weak graft-to-bone integration. Therefore, replicating the native biological, structural, and mechanical features of the tendon-to-bone interface is the idealized scenario for designing tissue engineered constructs for enthesis repair. Advanced techniques, for example, electrospinning and 3D printing, enable control of multiscale material properties and spatial deposition of mineral and biochemical patterning akin to the native enthesis. Development and investigation of materials that functionally restore the structural, cellular, and mechanical properties of the enthesis are needed to improve the repair of this unique musculoskeletal organ.

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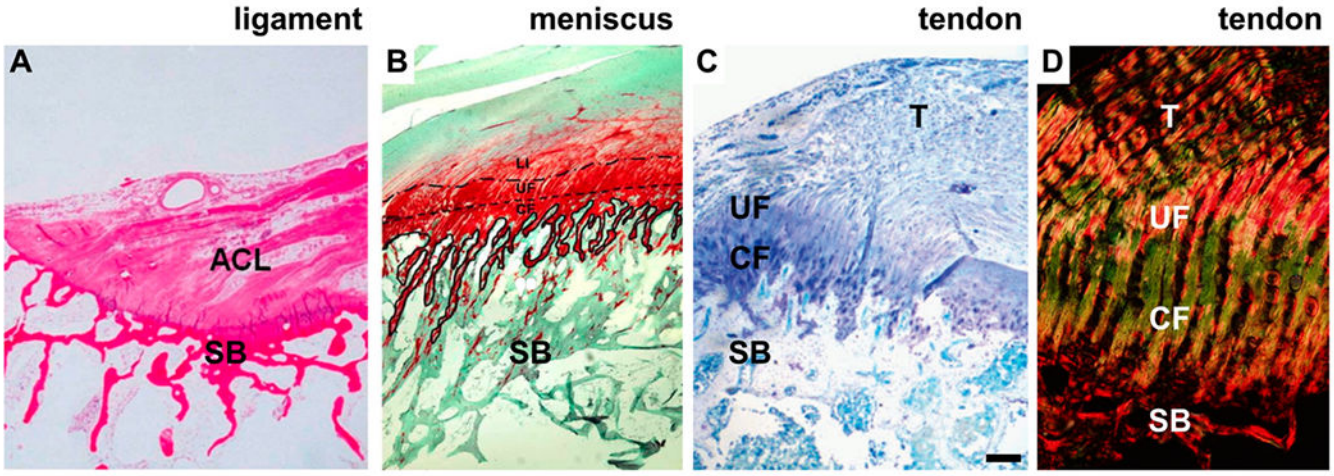

Figure 1.

Enthesis of the anterior cruciate ligament (ACL), meniscus, and rotator cuff tendon, shown histologically. SB = subchondral bone, CF = calcified fibrocartilage, UF = unmineralized fibrocartilage, and T = tendon. (A) Histology of the ACL enthesis at the tibial attachment, stained using hematoxylin and eosin.[43] (B) Histology of the meniscal enthesis, stained using Safranin O,[44] highlights the fibrocartilage region rich in proteoglycan in red. The dashed line in B indicates the tidemark between SB and CF. (C and D) Histology of the supraspinatus enthesis of the rotator cuff tendon, stained using (C) toluidine blue[45] for enhanced metachromatic stain of the fibrocartilage (purple color) and (D) picrosirius red for enhanced birefringence under polarized light. Scale bar in C = 200 μm. The image in A was adapted with permission from ref 43. Copyright 2014 Sage. The image in B was adapted with permission from ref 44. Copyright 2008 Springer. The image in C was modified with permission from 45. Copyright 2011 International Bone and Mineral Society.

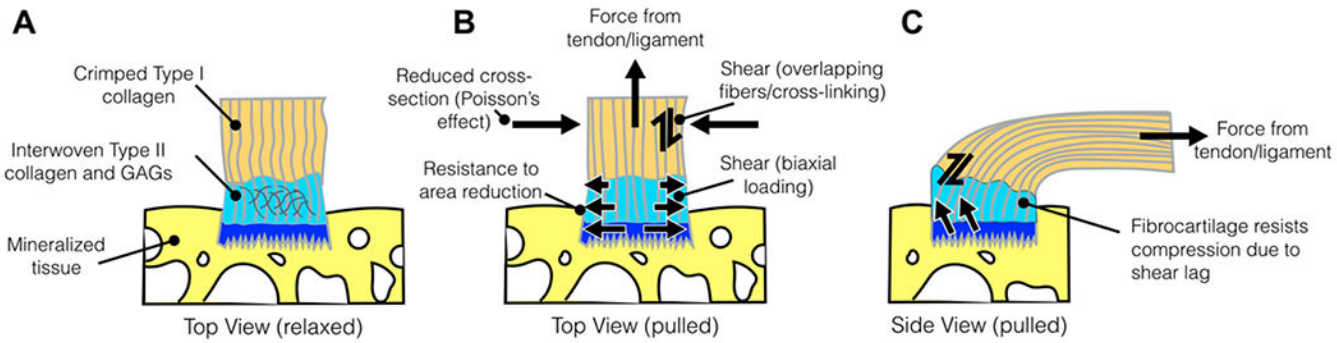

Figure 2.

(A–C) Tan coloration represents the tendon, light blue represents the uncalcified fibrocartilage (UF), dark blue represents the calcified fibrocartilage (CF), and yellow represents the bone. Forces applied to the enthesis, illustrated in both B (top view) and C (side view), are derived from tensile tendon/ligament force, compression, and shear.

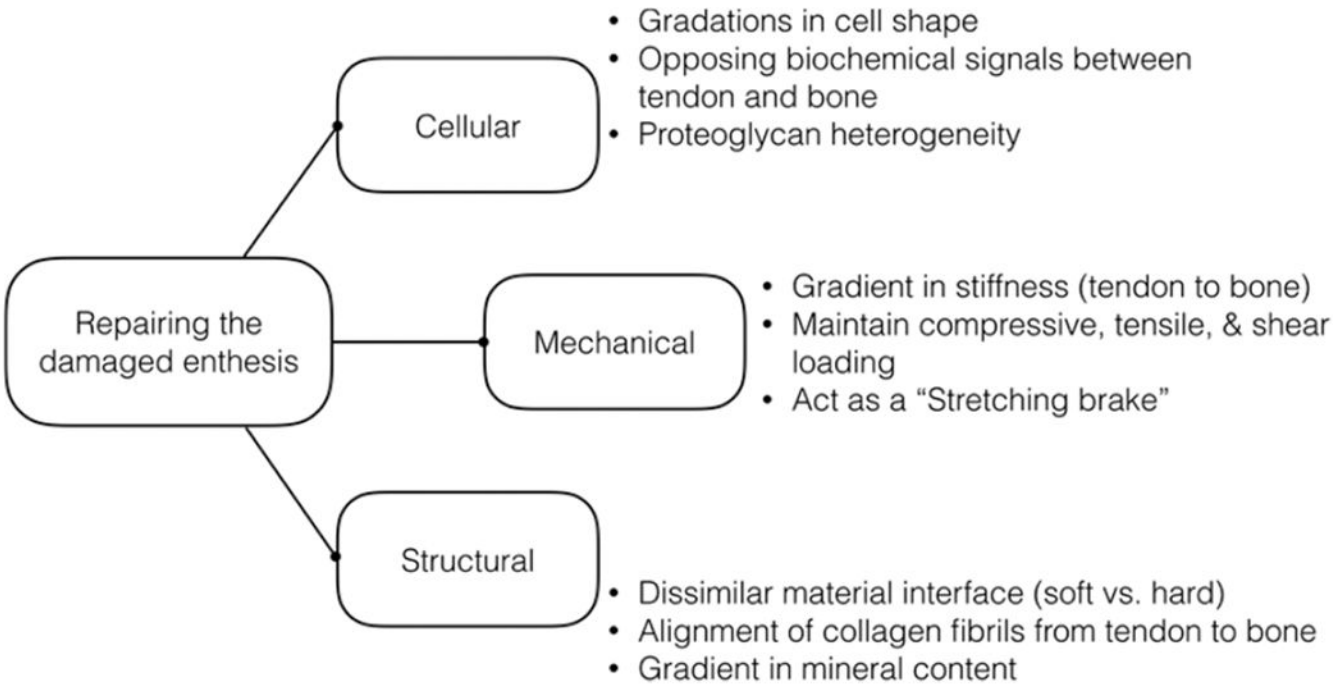

Figure 3.

Repairing the damaged enthesis requires design criteria and strategies aimed to mimic the native cellular, mechanical, and structural features of the enthesis.