Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biomedical Engineering

Achieving tendon enthesis regeneration across length scales

Ismael Bousso[1], Guy Genin[2] and Stavros Thomopoulos[1,3]

Abstract

Surgical reattachment of tendon to bone is a clinical challenge, with unacceptably high retear rates in the early period after repair. A primary reason for these repeated tears is that the multiscale toughening mechanisms found at the healthy tendon enthesis are not regenerated during tendon-to-bone healing. The need for technologies to improve these outcomes is pressing, and the tissue engineering community has responded with many advances that hold promise for eventually regenerating the multiscale tissue interface that transfers loads between the two dissimilar materials, tendon, and bone. This review provides an assessment of the state of these approaches, with the aim of identifying a critical agenda for future progress.

Addresses

[1] Department of Biomedical Engineering, Columbia University, New York, NY, USA

[2] Department of Mechanical Engineering & Materials Science, Washington University, St. Louis, MO, USA

[3] Department of Orthopaedic Surgery, Columbia University, New York, NY, USA

Corresponding author: Thomopoulos, Stavros (sat2@columbia.edu)

Current Opinion in Biomedical Engineering 2024, **31**:100547

This review comes from a themed issue on **Tissue Engineering & Regenerative Medicine: Tendon and ligament regeneration**

Edited by **Melissa Knothe Tate** and **Helen Lu**

Received 7 September 2023, revised 25 April 2024, accepted 17 May 2024

Available online xxx

https://doi.org/10.1016/j.cobme.2024.100547

Keywords

Enthesis, Tendon, Rotator cuff, Multiscale, Biomechanics, Tissue engineering.

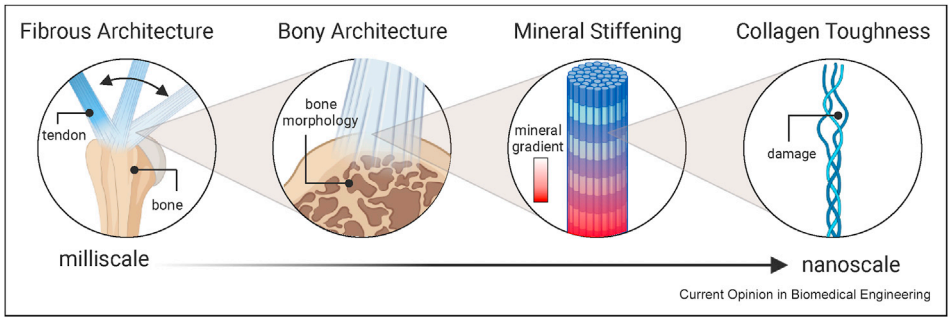
Introduction

Tendons and ligaments attach to bone across the enthesis, a multiscale tissue interface that transfers loads between these two dissimilar materials, and that dissipates stress concentrations. The enthesis achieves these functions through a fibrous material system with spatial, functional gradients in composition, structure, and mechanical properties (Figure 1) [1]. This functional grading is continuous, with smooth transitions across the four histological regions that were once thought to be distinct zones: tendon/ligament proper, unmineralized fibrocartilage, mineralized fibrocartilage, and bone [2]. Histologically, cellular morphology, matrix composition and structure, and mineral content differ substantially across the enthesis [2]. The anisotropic tendinous and ligamentous regions are composed of elongated fibroblasts embedded in a highly aligned collagen I matrix. The fibrocartilaginous regions, consisting of unmineralized and mineralized portions, contain ovoid fibro-chondrocytes and hypertrophic chondrocytes in a matrix rich in proteoglycans and collagen type II. Bone mainly comprises collagen I fibers and a mineralized matrix populated with osteoblasts, osteocytes, and osteoclasts [2]. Collagen alignment varies across the enthesis, from highly aligned in the tendon, to less aligned in the fibrocartilaginous regions, to an intermediate level with increasing mineralization near the bone.

At the macroscale, tendons function as biological springs with non-linear stress–strain curves and a modulus of ~200 MPa under tension [1]. In contrast, bone is relatively brittle with a modulus of ~20 GPa [3]. Connecting two tissues with such a high mismatch in mechanical characteristics is a challenge, as stress concentrations arise at their interface [1,3]. The enthesis solves this mechanical problem through a multiscale set of mechanisms, including macroscale optimization of attachment footprint area, microscale spatial gradients in mineral content, and nanoscale interactions between collagen molecules and mineral crystals. This robust attachment is so effective that failure rarely occurs within the enthesis: in adults, rupture tends to occur at the tendon/ligament midsubstance, while bony avulsion is typical in adolescents [1,4].

So why do so many older people have chronic problems in tissues such as the rotator cuff? The answer lies in the inability of the enthesis to regenerate: the intricate, multiscale mechanical mechanisms that optimize enthesis mechanics are not recreated after surgical repair of tendon to bone [5,6]. The lack of regeneration results in unacceptable post-surgical outcomes. Focusing on the rotator cuff, 20% of young patients and up to 94% of elderly patients experience re-tearing after surgery [1,5,6]. Most of these failures occur as sutures pull out of the repaired tendon in the days immediately following repair [7]. The healing process produces scar tissue and poor mechanical behavior, rather than regenerating a healthy enthesis [1,3–5]. Unfortunately,

Figure 1



Multiscale mechanisms of attachment between tendon and bone.

non-surgical therapies have also failed to improve outcomes.

Our current understanding of multiscale enthesis mechanics and observations of poor tendon-to-bone healing motivates the need for regenerative therapies. These therapies must consider recreating the macro-, micro-, and nano-scale features of the native healthy enthesis. This includes the aforementioned, continuous spatial gradient in composition, structure, and cell phenotype, necessary for mitigating stress concentrations and maintaining a functional attachment. Tissue engineering techniques must therefore consider implementing these gradients, either directly in the biomaterial or through the promotion of cellular activity. The purpose of this short review is to summarize the multiscale biomechanics of the tendon enthesis and consider recent progress in the development of biomaterial- and cell-focused strategies used to promote regeneration at each scale.

Macroscale enthesis mechanics and regeneration

As tendon inserts into bone, it exhibits a splayed geometry that avoids the Williams-type free edge singularity associated with the bi-material interfaces. The Williams free edge singularity, a stress concentration that arises at the intersection of an elastic material and a rigid substrate, is a power-law singularity at which stresses approach infinity as the distance from the edge approaches zero [8]. By incorporating a splayed geometry, entheses can eliminate this singularity, and can also distribute stresses more evenly across the interface in a way that is highly conserved across species and muscle sizes [9]. Stress concentrations depend strongly upon fiber orientations and recruitment, and these, too, appear optimized across different entheses to improve attachment mechanics [5]. Mouse rotator cuff enthesis experiments and computational models showed that different entheses enable differential reorientation and recruitment fibers to balance strength and toughness across a wide range of shoulder motion, with tradeoffs seeming to emphasize toughness over strength in the extremes of these motions.

During healing, macroscale enthesis features are not regenerated, with weak isotropic scar tissue replacing the native enthesis. Few tissue engineering strategies have focused on recreating macroscale features such as a splayed geometry or complex fibrous architecture. Approaches have included the use of decellularized entheses as scaffolds, 3D-printed tendon-bone or ligament-bone structures, and multiphasic biomaterial scaffolds [10–13]. Each of these has shown some success in promoting the desired cellular responses and, in some cases, enhanced mechanical properties in vitro; however, *in vivo* results have been disappointing. In many cases, approaches have failed because of the mismatches in length scales at the tissue interface. Repair techniques have also considered macroscale attachment mechanisms. Suturing techniques and tissue grafts remain the gold standard for clinical repair of connective tissue interfaces in rotator cuff, anterior cruciate ligament, and Achilles tendon, leaving much room for tissue-engineered strategies that improve clinical efficacy.

Microscale enthesis mechanics and regeneration

At the microscale, the tendon enthesis consists of a spatially graded, fibrous architecture of collagen fiber bundles, with a monotonically increasing gradient of minerals as it inserts into the bone. Recent work has described the three-dimensional arrangement of these fibers and the location of the mineral crystals. Collagen fibers that are predominantly aligned at the tendon end become less aligned through fibrocartilage, leading to a region between tendon and bone that is less stiff than either tendon or bone [3,14]. This region of compliant tissue contains mineral at concentrations just below the steric percolation threshold, i.e., the point at which the mineral phase forms a mechanically contiguous

structure across the collagen fibril. These mineral platelets may also absorb energy via their mobility, further contributing to the overall toughness of the enthesis [15]. Additional mechanisms of toughening at this scale include the interdigitation of fibers into the bone, which may increase load-bearing area and introduce stress concentrations that toughen the enthesis through stochastic failure [16].In porcine entheses, tendon fibers uncoil into thinner fibers at angles of up to 15° before interdigitating into bone over a length scale of ~500 μm^3, again showing the compliant toughening band between tendon and bone seen previously in human shoulders [1,9,14]. This transition has been confirmed through numerous modeling studies that address factors such as the relevance of transversely isotropic models, and the critical role of competing gradients of mineral percolation and fiber organization [1,17]. The percolation threshold is critical because it simultaneously marks the point at which mineralization starts to have a substantial effect on microscale fiber stiffness, and also the point at which it loses its mobility. At the microscale, levels of both mineral deposits and roughness of the bony end of the enthesis leads to microstructural heterogeneity that toughens the enthesis and distributes failure stochastically at the macroscale, resulting in toughening of the insertion [15,18].

This microscale structure is not regenerated upon healing or repair, and its origins in development are thus of key importance. The microscale structure is built and maintained by a distinct population of cells, with a gradient in cellular phenotype from tendon to bone. This arises from the fetal origin of the enthesis, which is a distinct pool of cells that express the transcription factors Scleraxis (Scx) (associated with tendon development) and Sry-related HMG box 9 (Sox-9) (associated with cartilage development) [19]. Postnatally, these cells are activated by hedgehog (Hh) signaling (leading to the expression of Gli1) to form and mineralize a fibrocartilaginous enthesis. This cell phenotype, when studied using scRNAseq and lineage tracing approaches, revealed six resident cell types, including enthesis progenitors and mineralizing chondrocytes, all derived from Gli1-lineage cells [20]. These Gli1-lineage cells demonstrate clonogenicity and multipotency and improved healing when delivered to injured entheses [20–24].

In light of these gradients, microscale-targeted therapies that enhance tendon-to-bone healing are particularly interesting. Although no tissue-engineered technologies have yet succeeded in fully reconstituting the tendon enthesis, many highly promising technologies can be found in the literature, with too many exciting advances to list in this short review. Representative advances include that of Zhu et al., who used a combination of transforming growth factor 1 (TGF-β1), insulin-like growth factor 1 (IGF-1), and parathyroid hormone (PTH) to promote proliferation and differentiation for improved microscale distributions of tenocytes [25]; this combination of growth factors showed synergistic effects, including integrated fibrocartilage formation and improved mechanical integrity. In other work, efforts at stimulating the Hedgehog (Hh) pathway, previously established as a key regulator of enthesis fibrocartilage formation during growth and development [26–29], have shown some efficacy. By genetically activating Smo in SmoCA mice and using Hh-agonist1.5 as a pharmacological stimulant, increased mineralized fibrocartilage formation and integration were seen in mice [26]. In a different approach, hydrogels were loaded with exosomes derived from kartogenin-preconditioned mesenchymal stem cells (MSCs) and used to enhance cartilage formation and collagen maturation for enthesis regeneration [30]. Similarly, hydrogels containing neonatal tendon progenitor cells delivered to the supraspinatus tendon increased the number of Sox9+ expressing fibrocartilage cells and reduced overall scar markers [31]. Several other approaches have delivered cells and biofactors to healing entheses in an effort to promote enthesis regeneration [10,32,33].

In addition to cells and biofactors, organoid technology holds promise for enthesis regeneration. Organoids can be used for disease modeling, drug discovery, and personalized medicine. Although an enthesis-specific organoid has not yet been developed, cell-loaded hydrogel microspheres have been used to construct osteo-callus organoids that, when treated with chondrogenic differentiation media, exhibit endochondral ossification and upregulation of hypertrophic and osteogenic markers that are also found in the developing enthesis during mineralization [34]. Similarly, cartilage organoids have been generated using hydrogel microspheres [35], osteoinductive bioceramics [36], and 3D suspension systems [37]. These studies in bone and cartilage may possibly be adapted to develop enthesis organoids.

Despite some success in small animal models, cellular and biofactor approaches are not yet ready for clinical translation. A challenge facing the entire field when delivering growth factors is the avoidance of off-target effects. Many approaches exist to mitigate this challenge, but these stand as a barrier to patient safety and, before that, to FDA approval. Additionally, structural and material properties are not fully restored by any of these approaches, compared to intact nonsurgical controls. Additionally, these techniques are not yet optimized to overcome the primary source of enthesis re-

tearing, which is suture pullout in the early post-surgical window. These approaches may be further enhanced in future studies when used in combination with scaffolding materials to create hierarchical tissues. A particularly promising, recent approach to enthesis regeneration is to use textile-based technology, with structure and composition inspired by high resolution tissue imaging, to recreate the fibrous structure and attachment morphology of the enthesis in a way that can be augmented by bioactive molecules [38].

Nanoscale enthesis mechanics and regeneration

Tendon, ligament, and bone organize around hierarchical collagen structures, from tropocollagen molecules that self-assemble into collagen fibrils at the nanoscale to larger tissue structures at the macroscale [39]. Collagen molecules, consisting of three left-handed amino acid chains, are approximately 1.5 nm in diameter. At the fibril scale, collagen molecules have a D-band structure with a ~67 nm spacing filled with water and non-collagenous molecular cross-links [39], which allows collagen fibrils to absorb and dissipate mechanical stresses at the nanoscale level [40–42]. The D-band is composed of two regions called the overlap and gap regions. During mineralization, hydroxyapatite (HA) minerals replace water molecules within and atop the gap regions of fibrils. The mechanics of individual collagen fibers have been studied via microelectromechanical system (MEMS) devices, atomic force microscopy (AFM), and nanoindentation [39,43–45]. However, because no partially mineralized mammalian tissues have yet been tested, interpretations of these data in the context of mineralized tissues involve models of collagen-mineral arrangement in partially and progressively mineralized tissues [14,18], in conjunction with assumptions about nanoscale collagen-mineral interactions. For example, fibril mechanics studied using a full atomistic simulation model demonstrated that the presence of HA minerals partially reflects mechanical waves and increases stiffness at the interface between the overlap and gap regions. Additionally, increased loading speeds can reduce mechanical wave speed and Young's moduli due to the disruption of hydrogen-bond networks within fibrils [46]. However, these results are based on assumptions about fiber–mineral interactions. These interactions are as important as they are uncertain, because understanding how the nano-through macro-scale hierarchy of fibrils affect overall tissue behavior is pivotal for creating a bottom-up approach for enthesis regeneration.

The behavior of the collagen fibrils themselves is much better understood, with single fibril experiments and molecular dynamics simulations showing energy absorption even at the lowest levels of enthesis hierarchy [41,46,47]. Quasi-static behavior of collagen fibrils at low strains [48,49]is dominated by the interplay between molecular stretching and hydration, with straightening of hydrated collagen molecules dominating deformation mechanisms at strains below 10%, and stretching of hydrated collagen molecules dominating at higher strains and leading to strain-stiffening. In dehydrated fibrils, densely packed collagen molecules straighten with little stretching, resulting in moduli greater than hydrated collagen [49]. Collagen fibers undergo denaturation at high levels of strain that can lead to stress redistribution and fibril toughening [39,42,47]. The fibrils are also viscoelastic, with cyclically loaded experiments revealing strain-dependent energy dissipation [43–45] due to inelastic and viscous effects [39]. At low strains, uncoiling of tropocollagen molecules may occur; at intermediate strains, molecular sliding mechanism and associated loss of stiffness may dominate; at higher strains, a steady-state energy dissipation is present, with the collagen backbone stretching and molecular sliding restricted by crosslinks [35–43]. Viscoelastic behavior is evident in wave propagation studies of dehydrated and hydrated collagen molecules, with higher energy dissipation transverse to the fiber direction under hydrated conditions [41]. These experimental and modeling studies reveal strain-rate dependent, energy-absorptive behavior of collagen fibrils that can potentially create spatial heterogeneity in tangent modulus that can lead to further energy dissipation across hierarchies. However, the detailed nature of these interactions is still somewhat speculative due to challenges associated with multiscale experiments and simulations, and the role of partial mineralization remains uncertain.

Despite these uncertainties, a number of promising nanomaterials exist for promoting enthesis regeneration. These include both natural and synthetic approaches. Natural materials such as fibrin, collagen, silk fibroin, chitosan, and bioactive materials have been used due to their excellent biocompatibility and their fibrous nature, with fiber dimensions representative of those in the healthy enthesis. However, these materials often exhibit substantially lower strength, stiffness, and toughness than the native enthesis. As reviewed extensively elsewhere [50,51], approaches to synthesize functional fibrous materials include electrospinning, which creates submicron fibers by extruding polymer solution onto a collector, and bioprinting [52–54], which creates micron-scale features through layer-by-layer deposition of living cells and biomaterials [53–55]. Despite their attractive nanoscale features, electrospun nanofibrous scaffolds lack the strength needed for tendon enthesis applications. Efforts to address this shortcoming include

composite PEEUU/gel patches comprised of poly(-ether−ester−urethane)urea (PEEUU) and gelatin [56], cross-linked using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide and grafted with kartogenin for induction of chondrocytes. Such dual-network composite materials increase toughness and recapitulate nonlinear stress−strain behavior, and have certain enthesis-like features such as enhanced collagen organization and promotion of chondrocyte-like cells. Grafting of bioactive molecules onto nanofibers can also guide the differentiation of stem cells and the production of matrix proteins, as with silk-fibroin nanofibrous scaffolds with simulated body fluid (SBF) and mineral gradients [56], which can enhance and strengthen tendon-bone integration. Nanoscale features are possible in bioprinting using controlled diffusion, but this is limited to developing nanoscale spatial gradients and topography. Although the literature contains many such examples of successes in recapitulating key nanoscale details, generating a scaffold that can guide nanoscale enthesis repair while stabilizing the attachment mechanically during the healing phase remains an elusive goal.

Conclusions

The healthy tendon enthesis overcomes the mechanical challenge of attaching two materials with a high mechanical mismatch using hierarchical and multiscale mechanisms that rarely fail. In cases when enthesis or soft tissue injury occurs, both surgical and natural healing lead to scar tissues with properties that are inferior to the natural enthesis. Tissue-engineered approaches across the nano-through macroscales show promise for regenerating key features, but critical challenges remain, including localizing soluble factors, guiding the hierarchical regeneration of the enthesis, and providing the healing surfaces with long-term strength while simultaneously preventing the most common source of post-surgical failure, suture pull-out, in the days following repair.

Author contributions

IB, GG, and ST drafted and edited the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stavros Thomopoulos reports financial support was provided by National Institutes of Health. Guy Genin reports financial support was provided by National Science Foundation. Guy Genin reports financial support was provided by National Institutes of Health. Ismael Bousso reports financial support was provided by National Institutes of Health.

Acknowledgments

This work was supported by the National Institutes of Health (R01 AR080924, R01 AR080717, R01 AR077793), and the National Science Foundation (OIA-2219142, CMMI-1548571, DMR-2105150).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. ** Golman M, *et al.*: **Toughening mechanisms for the attachment of architectured materials: the mechanics of the tendon enthesis**. *Sci Adv* 2021, **7**:5584.
Golman et al. demonstrated several multiscale load transfer mechanisms at the enthesis that control trade-offs between strength and toughness, including macroscale collagen fibrous architecture, milli-scale load-sharing, and nanoscale protein denaturation. In vivo mouse models of pathology revealed that the loading environment drove architectural adaptations that optimized these multi-scale trade-offs.

2. Luo W, *et al.*: **Advanced strategies for constructing interfacial tissues of bone and tendon/ligament**. *J Tissue Eng* 2022, **13**.

3. ** Rossetti L, *et al.*: **The microstructure and micromechanics of the tendon−bone insertion**. *Nat Mater* 2017, **16**:664−670.
The authors observed that fibers within the enthesis behaved as a continuous network at the microscale to resist load. Tendon fibers transitioned into thinner interface fibers, splaying outwards before attaching to bone along an interdigitated line, resulting in a robust attachment. The mechanical response of this region was heterogeneous and angle dependent. Graded material properties and networked structures contributed to reduced stress concentrations and increased toughness.

4. Weiss JM, Arkader A, Wells LM, Ganley TJ: **Rotator cuff injuries in adolescent athletes**. *J Pediatr Orthop Part B* 2013, **22**:133−137.

5. Long Z, *et al.*: **Age-related cellular and microstructural changes in the rotator cuff enthesis**. *J Orthop Res* 2022, **40**:1883−1895.

6. Paxton ES, *et al.*: **Clinical and radiographic outcomes of failed repairs of large or massive rotator cuff tears: minimum ten-year follow-up**. *J Bone Jt Surg Am* 2013, **95**:627−632.

7. Cummins CA, Murrell GAC: **Mode of failure for rotator cuff repair with suture anchors identified at revision surgery**. *J Shoulder Elb Surg* 2003, **12**:128−133.

8. Williams ML, Pasadena C: *Stress singularities resulting from various boundary conditions in angular corners of plates in extension*. 1952.

9. Saadat F, Deymier AC, Birman V, Thomopoulos S, Genin GM: **The concentration of stress at the rotator cuff tendon-to-bone attachment site is conserved across species**. *J Mech Behav Biomed Mater* 2016, **62**:24−32.

10. Cao Y, *et al.*: **Three-dimensional printed multiphasic scaffolds with stratified cell-laden gelatin methacrylate hydrogels for biomimetic tendon-to-bone interface engineering**. *J Orthop Transl* 2020, **23**:89−100.

11. * Chen C, *et al.*: **Functional decellularized fibrocartilaginous matrix graft for rotator cuff enthesis regeneration: a novel technique to avoid in-vitro loading of cells**. *Biomaterials* 2020, **250**, 119996.
CXCR4 + SMSCs were isolated from rat synovium and sorted using CXCR4 as a marker. These cells exhibited distinct differentiation capabilities compared to BMSCs, with greater potential for chondrogenesis and adipogenesis. The study introduced C-SDF-1α/BDFM, a biomaterial scaffold that effectively recruited CXCR4 + SMSCs and promoted their chondrogenic differentiation in vitro. In vivo, C-SDF-1α/BDFM facilitated CXCR4 + SMSC recruitment to the healing site of a rotator cuff injury, resulting in improved tissue regeneration and biomechanical strength.

12. Brown ME, Puetzer JL: **Driving native-like zonal enthesis formation in engineered ligaments using mechanical boundary conditions and β-tricalcium phosphate**. *Acta Biomater* 2022, **140**:700−716.

13. Pugliese E, *et al.*: **Development of three-layer collagen scaffolds to spatially direct tissue-specific cell differentiation for enthesis repair**. *Mater Today Bio* 2023, **19**, 100584.

14. Genin GM, *et al.*: **Functional grading of mineral and collagen in the attachment of tendon to bone**. *Biophys J* 2009, **97**: 976–985.

15. Lipner J, Boyle JJ, Xia Y, Birman V, Genin GM, Thomopoulos S: **Toughening of fibrous scaffolds by mobile mineral deposits**. *Acta Biomater* 2017, **58**:492–501. https://doi.org/10.1016/J.ACTBIO.2017.05.033.

16. Hu Y, *et al.*: **Stochastic interdigitation as a toughening mechanism at the interface between tendon and bone**. *Biophys J* 2015, **108**:431–437.

17. Boys AJ, *et al.*: **Understanding the stiff-to-compliant transition of the meniscal attachments by spatial correlation of composition, structure, and mechanics**. *ACS Appl Mater Interfaces* 2019, **11**:26559–26570.

18. Liu Y, *et al.*: **Modelling the mechanics of partially mineralized collagen fibrils, fibres and tissue**. *J R Soc Interface* 2014, **11**.

19. Huang AH, *et al.*: **Requirement for scleraxis in the recruitment of mesenchymal progenitors during embryonic tendon elongation**. *Development* 2019, **146**.

20. ** Fang F, Xiao Y, Zelzer E, Leong KW, Thomopoulos S: **A mineralizing pool of Gli1-expressing progenitors builds the tendon enthesis and demonstrates therapeutic potential**. *Cell Stem Cell* 2022, **29**:1669–1684.e6.
Single-cell RNA sequencing and lineage tracing were used to define the postnatal development of the mouse enthesis. Six enthesis cell types were identified along with transcription factor regulons and temporal regulation of differentiation. A unique population of Gli1+ entheiss stem cells were identified with therapeutic potential to regenerate the injured enthesis.

21. Fang F, Schwartz AG, Moore ER, Sup ME, Thomopoulos S: **Primary cilia as the nexus of biophysical and hedgehog signaling at the tendon enthesis**. *Sci Adv* 2020, **6**.

22. * Xiao H, *et al.*: **Mechanical stimulation promotes enthesis injury repair by mobilizing Prrx1+ cells via ciliary TGF-β signaling**. *Elife* 2022, **11**.
This study showed that mechanical stimulation can promote TGF-β1 expression and facilitate mobilization of Prx1+ cells, ultimately promoting the repair of enthesis injuries through ciliary TGF-β signaling. The research sheds light on the crucial role of mechanical cues in enthesis injury repair.

23. Chen C, *et al.*: **Book-shaped acellular fibrocartilage scaffold with cell-loading capability and chondrogenic inducibility for tissue-engineered fibrocartilage and bone-tendon healing**. *ACS Appl Mater Interfaces* 2019, **11**: 2891–2907.

24. Zhang T, *et al.*: **Single-cell RNA sequencing reveals cellular and molecular heterogeneity in fibrocartilaginous enthesis formation**. *bioRxiv* 2023, 2023.02.02.526768. https://doi.org/10.1101/2023.02.02.526768.

25. Zhu M, *et al.*: **Novel growth factor combination for improving rotator cuff repair: a rat in vivo study**. *Am J Sports Med* 2022, **50**:1044–1053.

26. ** Kamalitdinov TB, *et al.*: **Targeting the hedgehog signaling pathway to improve tendon-to-bone integration**. *Osteoarthr Cartil* 2023, **31**:1202–1213.
The authors revealed that Hh-related gene activity increases as attachments mature and during the healing process. By genetically and pharmacologically stimulating the Hh pathway, a role for this pathway was demonstrated for promoting bone marrow-derived progenitor cell proliferation and fibrocartilaginous attachment formation, ultimately improving integration strength.

27. Schwartz AG, Long F, Thomopoulos S: **Enthesis fibrocartilage cells originate from a population of Hedgehog-responsive cells modulated by the loading environment**. *Development* 2015, **142**:196–206.

28. Bangs F, Anderson KV: **Primary cilia and mammalian hedgehog signaling**. *Cold Spring Harb Perspect Biol* 2017, **9**.

29. Fang F, Sup M, Luzzi A, Ferrer X, Thomopoulos S: **Hedgehog signaling underlying tendon and enthesis development and pathology**. *Matrix Biol* 2022, **105**:87.

30. Cai J, *et al.*: **Exosomes derived from kartogenin-preconditioned mesenchymal stem cells promote cartilage formation and collagen maturation for enthesis regeneration in a rat model of chronic rotator cuff tear**. *Am J Sports Med* 2023, **51**:1267–1276.

31. Vervaecke AJ, *et al.*: **Tendon progenitor cells as biological augmentation improve functional gait and reduce scar formation after rotator cuff repair**. *J Shoulder Elb Surg* 2022, **31**: 2366–2380.

32. Chen C, *et al.*: **Engineering an enthesis-like graft for rotator cuff repair: an approach to fabricate highly biomimetic scaffold capable of zone-specifically releasing stem cell differentiation inducers**. *Bioact Mater* 2022, **16**:451–471.

33. Lee-Barthel A, Lee CA, Vidal MA, Baar K: **Localized BMP-4 release improves the enthesis of engineered bone-to-bone ligaments**. *Transl Sports Med* 2018, **1**:60–72.

34. Xie C, *et al.*: **High-efficient engineering of osteo-callus organoids for rapid bone regeneration within one month**. *Biomaterials* 2022, **288**, 121741.

35. Shen C, *et al.*: **Boosting cartilage repair with silk fibroin-DNA hydrogel-based cartilage organoid precursor**. *Bioact Mater* 2024, **35**:429–444.

36. Li ZA, *et al.*: **Articular tissue-mimicking organoids derived from mesenchymal stem cells and induced pluripotent stem cells**. *Organoids* 2022, **1**:135–148.

37. Crispim JF, Ito K: **De novo neo-hyaline-cartilage from bovine organoids in viscoelastic hydrogels**. *Acta Biomater* 2021, **128**: 236–249.

38. Ng JL, Knothe LE, Whan RM, Knothe U, Tate MLK: **Scale-up of nature's tissue weaving algorithms to engineer advanced functional materials**. *Sci Rep* 2017, **7**:40396.

39. Liu J, *et al.*: **Energy dissipation in mammalian collagen fibrils: cyclic strain-induced damping, toughening, and strengthening**. *Acta Biomater* 2018, **80**:217.

40. Alcântara ACS, Felix LC, Galvão DS, Sollero P, Skaf MS: **Devising bone molecular models at the nanoscale: from usual mineralized collagen fibrils to the first bone fibers including hydroxyapatite in the extra-fibrillar volume**. *Materials* 2022, **15**:2274.

41. Milazzo M, Jung GS, Danti S, Buehler MJ: **Wave propagation and energy dissipation in collagen molecules**. *ACS Biomater Sci Eng* 2020, **6**:1367–1374.

42. Zitnay JL, *et al.*: **Accumulation of collagen molecular unfolding is the mechanism of cyclic fatigue damage and failure in collagenous tissues**. *Sci Adv* 2020, **6**.

43. Yang F, *et al.*: **Nonlinear time-dependent mechanical behavior of mammalian collagen fibrils**. *Acta Biomater* 2023, **163**: 63–77.

44. Yang F, Das D, Chasiotis I: **Strain rate induced toughening of individual collagen fibrils**. *Appl Phys Letts* 2022, **120**, 114101.

45. Yang F, Das D, Chasiotis I: **Microscale creep and stress relaxation experiments with individual collagen fibrils**. *Opt Lasers Eng* 2022, **150**.

46. Milazzo M, Jung GS, Danti S, Buehler MJ: **Mechanics of mineralized collagen fibrils upon transient loads**. *ACS Nano* 2020, **14**:8307–8316.

47. Buehler MJ: **Nanomechanics of collagen fibrils under varying cross-link densities: atomistic and continuum studies**. *J Mech Behav Biomed Mater* 2008, **1**:59–67.

48. Ghodsi H, Darvish K: **Investigation of mechanisms of viscoelastic behavior of collagen molecule**. *J Mech Behav Biomed Mater* 2015, **51**:194–204.

49. Gautieri A, Vesentini S, Redaelli A, Buehler MJ: **Hierarchical structure and nanomechanics of collagen microfibrils from the atomistic scale up**. *Nano Lett* 2011, **11**:757–766.

50. Saveh-Shemshaki N,S, Nair L, Laurencin CT: **Nanofiber-based matrices for rotator cuff regenerative engineering**. *Acta Biomater* 2019, **94**:64–81.

51. Li Y, Mo Z, Wenzhuo Z, Jianying Y, Nan J: **Scaffold-based tissue engineering strategies for soft–hard interface regeneration**. *Regen Biomater* 2023, **10**.

52. Chen Z, *et al.*: **Synergistic effects of mechanical stimulation and crimped topography to stimulate natural collagen development for tendon engineering**. *Acta Biomater* 2022, **145**: 297–315.

53. Wang L, *et al.*: **Crimped nanofiber scaffold mimicking tendon-to-bone interface for fatty-infiltrated massive rotator cuff repair**. *Bioact Mater* 2022, **16**:149–161.

54. Hosseinian H, *et al.*: **An origami-based technique for simple, effective and inexpensive fabrication of highly aligned far-field electrospun fibers**. *Sci Rep* 2023, **13**:1–15.

55. Lei T, *et al.*: **Biomimetic strategies for tendon/ligament-to-bone interface regeneration**. *Bioact Mater* 2021, **6**:2491–2510.

56. Chen C, *et al.*: **A reinforced nanofibrous patch with biomimetic mechanical properties and chondroinductive effect for rotator cuff tissue engineering**. *Mater Today Chem* 2022, **23**, 100655.