

Review

# Ligament Cell Biology: Effect of Mechanical Loading

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## Key Words

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## Abstract

Ligaments are biomechanically specialized connective tissues that maintain joint stability and guide motion under complex loading conditions. At the cellular and molecular levels, ligament homeostasis is governed by fibroblast-like cells (ligamentocytes) embedded in an intricately organized ECM composed predominantly of type I collagen, with contributions from type III collagen, elastin, proteoglycans, and glycoproteins. These cells continuously sense and respond to mechanical stimuli—tension, compression, and shear—through mechanotransduction pathways involving integrins, focal adhesions, cytoskeletal remodeling, and mechanosensitive ion channels. Downstream signaling cascades, including MAPKs and PI3K/AKT, integrate biomechanical cues with growth factor and cytokine signaling to fine-tune gene expression, collagen fibrillogenesis, and ECM turnover. Distinct from tendons, ligaments must adapt to multidirectional loads, resulting in unique ECM compositions and cellular phenotypes. Appropriate mechanical loading maintains collagen alignment, promotes ECM integrity, and stabilizes the ligament cell phenotype. By contrast, insufficient or excessive load alters the molecular balance, triggering catabolic processes, inflammation, and disorganized ECM assembly. This delicate equilibrium also underlies the ligamentization observed in ACL graft remodeling, where controlled mechanical environments and molecular interventions accelerate the acquisition of ligamentous properties. Emerging insights into transcriptional and epigenetic regulation, growth factor-mediated cues, and cytokine-driven responses offer avenues to engineer ligament-like tissues and optimize recovery strategies. By leveraging molecular knowledge of cell–matrix interactions, growth factor profiles, and genetic/epigenetic modulators, clinicians and researchers can design tailored loading protocols, biomimetic scaffolds, and regenerative therapies. These approaches aim to restore ligament functionality, enhance graft integration, and prevent degenerative changes, ultimately improving patient outcomes in ligament injury repair and reconstruction.

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## Introduction

Ligaments are critical connective tissues that link bones and guide joint motion, withstanding complex, multidirectional mechanical loads that ensure joint stability and proper arthrokinematics [1–3]. At the cellular and molecular levels, ligament biology is distinguished by a specialized population of fibroblast-like cells, often termed ligament fibroblasts or ligamentocytes, that reside within a highly organized extracellular matrix (ECM) [4–7]. The ECM of ligaments is composed predominantly of type I collagen, which provides tensile strength, but also includes type III collagen, elastin, and an array of proteoglycans and glycoproteins that contribute to viscoelastic behavior and tissue compliance [8–12]. This diverse molecular environment allows ligaments to fulfill their demanding mechanical functions under various loading regimes [13–15].

Within the ECM milieu, ligament fibroblasts actively sense and respond to mechanical cues through mechanotransduction pathways [16–19]. Mechanical loading—whether tensile stretch during extension, shear forces during rotational movements, or transient compressive loads during joint impact—modulates a cascade of intracellular signals that ultimately influence gene expression, protein synthesis, and ECM remodeling [20–23]. At the molecular level, integrins—transmembrane receptors that anchor cells to collagen fibrils—serve as primary sensors of mechanical forces and transmit signals to focal adhesion complexes [24–26]. These complexes include cytoskeletal adaptor proteins, such as vinculin and talin, which link integrin receptors to the actin cytoskeleton [27–29]. Changes in tension and matrix stiffness alter integrin clustering and conformation, triggering the activation of focal adhesion kinase (FAK) and downstream signaling molecules including mitogen-activated protein kinases (MAPKs) and PI3K/AKT pathways [30–33]. In parallel, ligament fibroblasts detect mechanical perturbations via mechanosensitive ion channels, stretch-activated receptors, and the deformation of primary cilia, all of which converge upon intracellular signal transduction networks [34–37]. These signals dictate the transcriptional regulation of structural proteins (e.g., collagen I, collagen III), proteoglycans (decorin, biglycan), and matrix-remodeling enzymes (e.g., matrix metalloproteinases), as well as growth factors and cytokines that modulate cellular proliferation, differentiation, and ECM turnover [38–42]. Additionally, dynamic changes in ECM composition influence ligand binding and growth factor sequestration, further refining the molecular interplay between cells and their surrounding matrix [43–45]. This intricate relationship ensures that ligament fibroblasts continuously adapt their molecular and cellular states in response to the mechanical environment [46–48]. When subjected to appropriate levels of physiologic load, these cells maintain homeostasis—preserving proper collagen fibril alignment, ECM integrity, and tissue mechanical properties [49–51]. Conversely, deviations in loading conditions, such as excessive strain or prolonged unloading, can elicit maladaptive cellular responses, leading to ECM disorganization, altered collagen fiber composition, increased proteolytic enzyme activity, and a shift towards a more fibrocartilaginous phenotype [52–55]. Such maladaptive changes underlie ligament degeneration, impaired healing, and compromised mechanical function following injury [56–58].

A nuanced understanding of ligament cell mechanobiology is, therefore, pivotal for informing clinical strategies aimed at restoring normal tissue function after injury, enhancing rehabilitation protocols, and advancing tissue engineering approaches that mimic the native mechanical microenvironment [59–61]. By manipulating molecular signaling pathways, fine-tuning mechanical stimulation parameters, and leveraging emerging biotechnologies—such as growth factor delivery, gene editing, or biomimetic scaffolds—it may be possible to direct ligament fibroblasts toward desirable phenotypes [62–64]. Ultimately, improving our knowledge of the molecular and cellular responses to mechanical loading will contribute significantly to the prevention of ligament injuries, the optimization of post-injury treatment, and the successful engineering of functional ligamentous tissues [65–67].

## Molecular and Cellular Differences Between Ligaments and Tendons

While ligaments and tendons share fundamental structural and hierarchical organization—both are dense, collagen-rich connective tissues connecting musculoskeletal components—their molecular profiles and cellular phenotypes diverge to accommodate distinct biomechanical roles (Table 1). Tendons typically transfer unidirectional tensile loads from muscle to bone, resulting in a highly aligned, parallel array of type I collagen fibers with minimal elastin and relatively sparse proteoglycan content [68–71]. This predominance of type I collagen, coupled with low levels of glycosaminoglycans (GAGs), reflects an adaptation to resist tensile loading along a single predominant axis. Ligaments, in contrast, withstand more complex, multidirectional mechanical environments associated with joint stabilization. As a result, ligament ECM composition frequently includes a higher proportion of type III collagen interspersed among the predominant type I collagen fibrils, as well as greater quantities of elastin and small leucine-rich proteoglycans (SLRPs) such as decorin, biglycan, fibromodulin, and lumican [72–74]. These molecules modulate collagen fibrillogenesis, influence fibril diameter distribution, and contribute to the viscoelastic properties needed to endure variable loading states. The presence of elastin and a richer array of proteoglycans imparts ligaments with greater compliance and an enhanced ability to absorb and dissipate complex stresses, improving joint stability while allowing controlled mobility. At the cellular level, ligament fibroblasts (ligamentocytes) differ from tendon fibroblasts (tenocytes) in their gene expression patterns and responsiveness to mechanical signals [75–77]. Ligament cells express a distinct repertoire of integrins, including integrin subunits that may preferentially bind to ECM components enriched in ligaments,

**Table 1.** This table highlights how tendons and ligaments, despite both being collagen-rich connective tissues, differ in ECM composition, mechanical properties, cellular phenotypes, integrin expression, and transcriptional/epigenetic regulation. These distinctions form the basis for targeted regenerative medicine approaches that manipulate molecular and cellular factors to steer tendon grafts and engineered constructs toward a ligamentous phenotype, ultimately improving clinical outcomes in ligament repair and reconstruction

Component	Tendons	Ligaments	References
Primary Function	Transmit unidirectional tensile loads from muscle to bone	Stabilize joints under complex, multidirectional loads	[68–71], [72–74]
Collagen Composition	Predominantly type I collagen in parallel, aligned fibers; low elastin and proteoglycan content	Higher proportion of type III collagen intermixed with type I; increased elastin and proteoglycans (decorin, biglycan, fibromodulin, lumican)	[68–74]
ECM Organization & Fibrillogenesis	Uniform, linear collagen fibrils optimized for tension along one axis	More variable fibril diameter distribution; ECM modulated by SLRPs to handle complex loading states	[72–74]
Viscoelastic & Mechanical Properties	Primarily tensile strength and stiffness; minimal compliance	Greater compliance and viscoelasticity; enhanced ability to absorb and dissipate complex stresses	[72–74]
Glycosaminoglycans (GAGs) & Proteoglycans	Relatively sparse GAGs and proteoglycans	Richer array of proteoglycans (e.g., decorin, biglycan) contributing to viscoelasticity	[72–74]
Elastin Content	Minimal elastin, mainly linear collagen arrays	Higher elastin content for multidirectional load absorption and controlled mobility	[72–74]
Cellular Phenotype (Fibroblasts)	Tenocytes: specialized for uniaxial load; integrins tuned to linear collagen I matrices	Ligamentocytes: respond to complex loading; integrins favor binding to collagen III- and proteoglycan-enriched matrices	[75–77]
Gene Expression & Integrin Profile	Tendon cells rely on integrins matching linear collagen I; lower elastin and proteoglycan gene expression	Ligament cells express integrins and genes favoring elastin, proteoglycans, and ECM remodeling under complex loads	[75–77]
Growth Factors & Cytokines	Growth factor signals adjust collagen synthesis but tend to maintain a more linear, tensile ECM	Distinct growth factor (e.g., TGF- $\beta$ , FGF, CTGF) and cytokine profiles that support remodeling, elastin production, and proteoglycan synthesis to handle multidirectional stresses	[78–80]
Transcription Factors (Scx, Mxk)	High Scx activity in tendon development reinforces linear collagen networks; Mxk expression less emphasized	Both Scx and Mxk influence ligamentous phenotype; differential expression of Mxk and GDFs encourages elastin and proteoglycan production for ligament ECM	[78–80]
Epigenetic Regulation (miRNAs, Chromatin)	Tendon phenotype maintained by epigenetic factors aligned with tensile homeostasis	Epigenetic mechanisms (miRNAs, chromatin modifiers) adapt gene expression to match ligamentous environments, ensuring ligament fibroblasts resist drift towards a tendinous identity	[78–80]
Clinical & Engineering Implications	When using tendon grafts for ligament reconstruction (e.g., ACL), the graft must be “ligamentized” to acquire appropriate molecular and mechanical properties	Adjusting mechanical loading regimes, delivering specific growth factors, and modulating transcription/epigenetic regulators can shift tendon-like cells toward a ligamentous phenotype, improving graft integration and joint biomechanics	[78–80]

influencing downstream signaling pathways that guide cytoskeletal organization and ECM remodeling. For instance, certain  $\alpha$ - and  $\beta$ -integrin subunits may be upregulated in ligament fibroblasts to facilitate adhesion to collagen III- and proteoglycan-rich matrices, while tendon cells rely more heavily on integrins tuned to linear collagen I networks. Additionally, ligament fibroblasts produce higher levels of elastin and glycoproteins that enhance tissue compliance and enable load distribution across multiple axes. Their secretory profile often includes distinct growth factors (e.g., TGF- $\beta$  isoforms, FGF, and CTGF) and cytokines that reflect the need for dynamic remodeling under complex loading conditions. These signaling molecules not only regulate collagen synthesis and degradation but also modulate matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), balancing anabolic and catabolic activities to maintain ligament homeostasis.

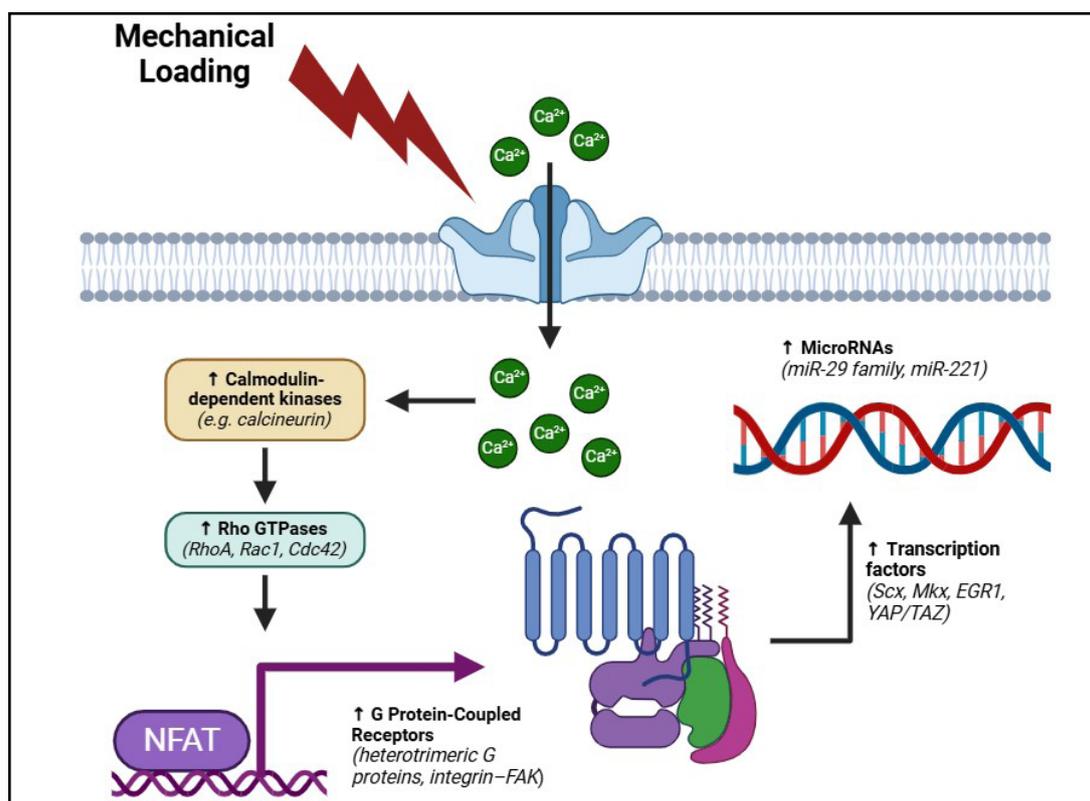
Key transcription factors such as scleraxis (Scx) and Mohawk (Mkx) are central to both tendon and ligament development, but subtle variations in their expression, as well as their interaction with cofactors like EGR1, GDFs, and other regulatory proteins, can steer cells toward a more ligamentous or more tendinous phenotype [78–80]. For example, differential expression levels of Mkx and GDFs might favor the production of elastin and proteoglycans characteristic of ligament ECM, while high Scx activity in concert with different co-regulators might reinforce a strictly linear collagen network more akin to tendon tissue.

Epigenetic regulators, including microRNAs and chromatin-modifying enzymes, may also differ between ligament and tendon fibroblasts, fine-tuning gene expression to match the unique mechanical and biochemical environments of each tissue. These epigenetic factors can govern the responsiveness to growth factors, integrin-mediated signaling, and cytoskeletal stress, ensuring that the cells retain their ligamentous identity and avoid drifting toward a tendinous phenotype—or vice versa.

Understanding these molecular and cellular distinctions is crucial for regenerative medicine and tissue engineering strategies. When using tendon grafts to reconstruct ruptured ligaments (e.g., the anterior cruciate ligament), it is essential that the transplanted tissue acquires ligament-like molecular attributes. By manipulating mechanical loading parameters, delivering specific growth factors, or modulating transcriptional and epigenetic regulators, it may be possible to guide tendon cells toward a ligament-like phenotype. This level of molecular control can improve graft integration and ultimately enhance clinical outcomes after ligament reconstruction procedures.

## Mechanotransduction Pathways in Ligament Cells

Mechanotransduction—the process by which ligament fibroblasts convert mechanical stimuli into biochemical and genetic responses—underpins the dynamic remodeling and maintenance of connective tissues subjected to complex, multiaxial loads [81–83] (Fig. 1). In ligaments, this involves a finely orchestrated network comprising integrin receptors, mechanosensitive ion channels, G protein-coupled receptors (GPCRs), primary cilia, cytoskeletal filaments, and multiple downstream molecular effectors. Collectively, these sensors and signaling hubs regulate the synthesis, organization, and turnover of the extracellular ECM. Additionally, specific genetic variants such as R2482H can modify key components of calcium-dependent signaling via calcineurin—sometimes referred to as “calcineurin 3”—and transcription factors of the NFATC family, imparting unique susceptibility or adaptive responses in ligament tissues. Integrins, which exist as transmembrane  $\alpha/\beta$  heterodimers, physically couple ligament fibroblasts to collagen fibrils, proteoglycans, and glycoproteins within the ECM [84–86]. Mechanical strain alters the conformation and clustering of integrin subunits (e.g.,  $\alpha5\beta1$ ,  $\alpha v\beta3$ ), triggering the recruitment of focal adhesion proteins such as talin, vinculin, paxillin, and  $\alpha$ -actinin to nascent adhesion complexes [87–89]. Upon integrin activation, FAK undergoes phosphorylation, creating scaffolding sites for Src family kinases and other intermediates that converge on MAPK (ERK, JNK, p38) and PI3K/AKT pathways [30–32] [90–92]. ERK commonly mediates proliferation and collagen-



**Fig. 1.** Graphical representation of ligament mechanosensing. This Fig. illustrates the principal mechanosensitive pathways that operate within ligament fibroblasts following mechanical loading. At the cell–ECM interface, mechanical strain on integrins (e.g.,  $\alpha 5\beta 1$ ,  $\alpha v\beta 3$ ) triggers clustering of focal adhesion proteins—such as talin, vinculin, paxillin, and  $\alpha$ -actinin—thereby activating and phosphorylating focal adhesion kinase (FAK). In turn, FAK provides a platform for Src kinases and adaptors that merge into two major downstream pathways: the MAPK cascade (ERK, JNK, p38) and the PI3K/AKT axis. ERK drives cell proliferation and collagen gene expression, whereas JNK and p38 mediate cytoskeletal remodeling and MMP regulation. The PI3K/AKT pathway orchestrates cell survival signals and modulates collagen deposition, proteoglycan assembly, and fibril alignment. Meanwhile, mechanosensitive ion channels (e.g., TRPV4 and PIEZO1) detect membrane deformation and facilitate rapid  $\text{Ca}^{2+}$  influx, activating calmodulin-dependent kinases or phosphatases (e.g., calcineurin). Elevated intracellular  $\text{Ca}^{2+}$  also influences Rho GTPases (RhoA, Rac1, Cdc42), promoting changes in actin filament organization and focal adhesion architecture. These modifications alter nuclear shape, impacting gene accessibility and epigenetic regulation. Within the calcium signaling branch, calcineurin dephosphorylates NFATC family members (e.g., NFATC1, NFATC2, NFATC3), enabling their nuclear translocation. The R2482H variant can affect calcineurin’s sensitivity to  $\text{Ca}^{2+}$ , thereby influencing NFATC-driven ECM gene expression and MMP activity. In addition to integrin and ion channel inputs, GPCR activation and primary cilia bending under mechanical load provide further layers of mechanosensing. Conformational shifts in GPCRs trigger heterotrimeric G proteins, reinforcing integrin–FAK and  $\text{Ca}^{2+}$  pathways. Primary cilia respond to fluid shear or matrix deformation by regulating intraflagellar transport, thereby modulating transcription factors including scleraxis (Scx), Mohawk (Mkx), and EGR1. Finally, mechanical signals converge at the transcriptional and epigenetic level, where factors such as Scx, Mkx, EGR1, and YAP/TAZ integrate cytoskeletal tension cues to control ECM-related gene programs. Epigenetic modifications (histone alterations, DNA methylation, microRNAs) refine collagen and MMP expression, while growth factors (TGF- $\beta$ , FGF, CTGF) further reinforce fibril assembly and homeostatic tissue remodeling.

related gene expression, while JNK and p38 act as stress-activated kinases, reorganizing the actin cytoskeleton and modulating MMPs. Meanwhile, PI3K/AKT coordinates cell survival and metabolic adaptation, allowing ligament fibroblasts to calibrate collagen deposition, proteoglycan assembly, and fibril alignment according to the mechanical demands imposed

on the tissue.

Mechanosensitive ion channels—including members of the TRP (Transient Receptor Potential) superfamily (e.g., TRPV4) and PIEZO channels (e.g., PIEZO1)—translate physical deformation of the cell membrane into ionic fluxes [93–95]. When stretched or subjected to fluid shear, these channels open and permit rapid influx of ions, predominantly  $\text{Ca}^{2+}$ , into the cytoplasm. Intracellular calcium elevation activates calmodulin-dependent kinases or phosphatases (including calcineurin), which then modulate a host of transcriptional regulators [96–98]. Increased cytosolic  $\text{Ca}^{2+}$  also impacts the Rho family of GTPases (RhoA, Rac1, Cdc42), controlling actin stress-fiber formation and focal adhesion organization [99–100]. These cytoskeletal reconfigurations affect nuclear geometry and chromatin accessibility, linking external mechanical cues to epigenetic modifications (e.g., histone acetylation, DNA methylation) and facilitating the loading-specific regulation of ECM-related genes. A key component of the calcium-mediated branch of mechanotransduction in ligament cells is calcineurin, a  $\text{Ca}^{2+}$ /calmodulin-dependent serine/threonine phosphatase that can be referred to in some contexts as “calcineurin 3.” Calcineurin dephosphorylates members of the NFAT (Nuclear Factor of Activated T-cells) family—such as NFATC1, NFATC2, and NFATC3—enabling their translocation to the nucleus [96–98]. Once inside the nucleus, these NFATC factors orchestrate the expression of ECM components, MMPs, and other remodeling proteins critical for ligament integrity. The R2482H variant, typically found in the regulatory subunit gene of calcineurin (PPP3R1), can alter the phosphatase’s sensitivity to  $\text{Ca}^{2+}$  levels, in turn affecting the magnitude or speed of NFATC activation. Such a variant may predispose certain individuals to differential ligament adaptations or altered healing outcomes, exemplifying how genetic polymorphisms intersect with mechanical loading to influence tissue phenotype [81–83]. Besides integrin and ion channel signaling, ligament fibroblasts can detect mechanical cues via GPCRs [101–102]. Under load, G protein-coupled receptors undergo conformational shifts that trigger heterotrimeric G proteins, adding an extra layer of molecular crosstalk with integrin-FAK signals and  $\text{Ca}^{2+}$ -mediated pathways. Moreover, primary cilia—antenna-like organelles comprising a microtubule core—may project from ligament fibroblasts into the pericellular matrix [103–104]. Bending of these cilia in response to fluid shear or ECM deformation modulates intraflagellar transport, influencing downstream signaling modules that converge on the nucleus to regulate transcription factors like Scx, Mxk, and EGR1. Defects in ciliary assembly or function can thus compromise ligament fibroblasts’ ability to sense and adapt to changing mechanical environments.

Tensile loading predominantly arises from joint movements that elongate the ligament, creating strain on collagen fibrils and associated focal adhesions. This strain alters the conformation and clustering of integrin subunits (e.g.,  $\alpha 5\beta 1$ ,  $\alpha v\beta 3$ ), triggering the recruitment of focal adhesion proteins such as talin, vinculin, paxillin, and  $\alpha$ -actinin to adhesion complexes [87–89]. These nascent adhesions activate FAK and Src family kinases, converging on MAPK (ERK, JNK, p38) and PI3K/AKT pathways [30–32] [90–92]. ERK mediates proliferation and collagen gene expression, while JNK and p38 reorganize the actin cytoskeleton and regulate matrix metalloproteinases (MMPs). The PI3K/AKT pathway coordinates cell survival and metabolic adaptation, ensuring that collagen deposition and fibril alignment match the tensile demands imposed on the tissue.

By contrast, compressive loading is often transmitted through regions near entheses (bone–ligament junctions) or via changes in tissue hydrostatic pressure and fluid flow within the ECM. Under compression, ligamentocytes may experience altered osmotic balances that activate mechanosensitive ion channels such as TRPV4, which is sensitive to volumetric changes and hydrostatic pressure [93–95]. The resultant influx of  $\text{Ca}^{2+}$  engages calmodulin-dependent kinases or phosphatases (e.g., calcineurin), further modulating Rho GTPase activity (RhoA, Rac1, Cdc42) and promoting cytoskeletal reorganization. These shifts in cytoskeletal architecture can ultimately influence nuclear shape, chromatin accessibility, and the expression of ECM proteins vital for accommodating compressive stress.

Meanwhile, shear loading arises from fluid movement across cell surfaces or from sliding between collagen fibers under torsional stresses. Shear forces can open mechanosensitive ion channels, including PIEZO (PIEZO1) and certain TRP family members, permitting rapid

ionic flux—primarily  $\text{Ca}^{2+}$ —into the cytoplasm [93–95]. Elevated  $\text{Ca}^{2+}$  activates calcineurin, thereby regulating NFATC-dependent transcription and other  $\text{Ca}^{2+}$ -responsive pathways [96–98]. In addition, shear stress can bend primary cilia, altering intraflagellar transport and triggering signaling cascades that converge on transcription factors like Scx, Mxk, and EGR1. Taken together, tensile, compressive, and shear inputs each engage overlapping yet distinct molecular circuits, enabling ligamentocytes to fine-tune ECM remodeling depending on the specific mechanical context.

At the transcriptional and epigenetic level, mechanical signals converge on factors such as Scx, Mxk, EGR1, and YAP/TAZ, each playing a distinct role in specifying ligament lineage and ECM gene expression [105–107]. Alterations in cytoskeletal tension may reshape the nuclear lamina and affect how these factors and their coactivators access DNA. Simultaneously, epigenetic mechanisms—including modifications of histone proteins (acetylation, methylation, phosphorylation), chromatin remodeling complexes, and the action of noncoding RNAs—further refine ligament gene regulation [108–109]. MicroRNAs (e.g., miR-29 family, miR-221) can dampen excessive collagen transcription or MMP production, optimizing matrix turnover [110–111]. Meanwhile, mechanical loading can liberate latent growth factors like TGF- $\beta$  or modulate the activity of FGF and CTGF, reinforcing collagen fibril assembly and crosslinking in tandem with integrin/ion channel signals [39–41] [112]. Such growth factor signaling synchronizes fibroblast proliferation, ECM deposition, and fibril organization to maintain structural homeostasis or promote repair following injury [113]. Hence, mechanotransduction in ligament cells emerges as an integrated molecular circuit wherein integrins, mechanosensitive ion channels, GPCRs, and primary cilia provide multifaceted sensing capabilities, while calcineurin–NFATC signaling, Rho GTPases, transcription factors, and epigenetic regulators translate these signals into coordinated ECM remodeling. Under physiological loads, these pathways uphold balanced collagen synthesis, tissue resilience, and functional stability. However, perturbed mechanical environments—whether from insufficient or excessive loading, acute trauma, or genetic factors like R2482H—can yield disorganized collagen architectures, delayed healing, or degeneration.

## Molecular Adaptations to Mechanical Loading

Ligament cells exhibit remarkable sensitivity to their mechanical environment, continually adjusting their molecular output in response to variations in load (Table 2). Under physiologic levels of cyclic tensile loading—mimicking normal joint motion—ligament fibroblasts maintain a tightly regulated equilibrium between ECM synthesis and degradation, ensuring that tissue remodeling aligns with functional demands [42–44, 114–116]. In this balanced state, integrin-mediated signaling and focal adhesion assembly foster robust cytoskeletal organization, sustaining the elongated, spindle-shaped morphology characteristic of healthy ligament cells. The resulting mechanical cues influence key transcription factors, including Scx and Mxk, which are central to specifying and preserving the ligamentous phenotype [45–48, 117–119]. By promoting the expression of collagen I and III, proteoglycans, and associated glycoproteins, physiologic loading reinforces tensile strength, elasticity, and structural integrity. At the molecular level, physiological loading also impacts the activity of signaling cascades such as the MAPK (ERK, JNK, p38) and PI3K/AKT pathways. These pathways converge on the nucleus, modulating transcription factor binding to gene promoters and enhancers, thereby enhancing the synthesis of ECM proteins that support tissue homeostasis [120–122]. Balanced loading ensures that the rates of collagen deposition, fibrillogenesis, and crosslinking match the tissue's mechanical requirements. Through the controlled production of proteoglycans like decorin and biglycan, ligament cells maintain appropriate collagen fibril spacing, contributing to optimized viscoelastic properties that resist dynamic and complex loading patterns [123–124].

In contrast, deviations from this equilibrium—either by mechanical underloading or excessive strain—shift the molecular landscape toward a catabolic, degenerative state.

**Table 2.** Table delineates the molecular and cellular responses of ligament cells under different mechanical loading modalities: Physiologic Loading, Insufficient Loading, and Excessive Loading. This table highlights key aspects such as signaling pathways, gene expression, ECM composition, and clinical implications, supported by references

Aspect	Physiologic Loading	Insufficient Loading	Excessive Loading	References
Mechanical Environment	Cyclic tensile strains mimicking normal joint motion	Mechanical unloading (e.g., immobilization)	Excessive, abnormal tensile strains	[42–44,114–116], [125–126], [133–135]
Integrin & Focal Adhesion Signaling	Maintained integrin clustering and stable focal adhesions; robust FAK activation	Decreased integrin clustering; destabilized focal adhesions; reduced FAK phosphorylation	Altered integrin signaling; potential focal adhesion disassembly	[125–126]
Cytoskeletal Organization	Robust cytoskeletal organization; elongated, spindle-shaped fibroblasts	Cytoskeletal disarray; altered nuclear shape	Disrupted cytoskeleton; potential shift toward fibrocartilaginous phenotype	[45–48,117–119]
Signaling Cascades (MAPK, PI3K/AKT)	Balanced activation of MAPK (ERK, JNK, p38) and PI3K/AKT pathways; supports ECM homeostasis and proper collagen fibrillogenesis	Reduced MAPK and PI3K/AKT signaling; downregulation of anabolic genes; upregulation of catabolic mediators	Enhanced catabolic signaling; increased MAPK and PI3K/AKT pathway activity leading to ECM degradation	[120–122], [133–135], [130–132]
ECM Protein Synthesis	Upregulation of collagen I & III; controlled production of proteoglycans (decorin, biglycan); optimized tensile strength and viscoelasticity	Downregulation of anabolic genes; increased collagen III relative to collagen I; disrupted fibril organization	Increased synthesis of MMPs; enhanced ECM degradation; altered expression of tenascin-C	[49–51,127–129], [123–124]
Gene Regulation (Scx, Mlx, miRNAs)	Stable expression of Scx and Mlx; miRNAs modulate ECM-related genes in a balanced manner	Downregulation of anabolic genes; upregulation of catabolic mediators; potential alteration of miRNA profiles	Altered expression of Scx and Mlx; miRNAs may promote collagen breakdown and MMP expression	[45–48,117–119], [133–135]
Inflammatory Mediators	Minimal pro-inflammatory cytokine release; growth factors (e.g., TGF- $\beta$ , FGF, CTGF) support anabolic ECM remodeling	Not directly addressed, but implied reduction in anabolic signaling	Increased IL-1 $\beta$ , TNF- $\alpha$ , and NO release; stimulation of MMPs; inflammatory feedback loop promoting ECM degradation	[52–54,130–132], [216–218], [80–82,213–215]
ECM Degradation vs. Synthesis	Equilibrium between ECM synthesis and degradation; balanced collagen deposition and crosslinking	Shift towards ECM degradation; reduced collagen synthesis; imbalance favoring catabolic activities	Dominance of ECM degradation over synthesis; imbalance leading to weakened ECM structure	[133–135], [49–51,127–129]
Transcription Factors	Activation of Scx and Mlx; promotion of ligamentous phenotype	Downregulation of Scx and Mlx; potential loss of ligamentous identity	Dysregulation of Scx and Mlx; induction of stress-responsive transcription factors	[45–48,117–119], [133–135]
Epigenetic Regulation	Balanced epigenetic modifications supporting ligamentous gene expression	Potential epigenetic dysregulation leading to reduced anabolic gene expression	Altered epigenetic marks promoting catabolic gene expression	[133–135]
Reactive Oxygen Species (ROS)	Controlled ROS levels; antioxidant defenses maintain cellular health	Not directly addressed	Elevated ROS levels; oxidative stress impairs protein folding, crosslink integrity, and cell membrane stability	[275–277], [313–315], [266–268]
Tissue-Level Outcome	Strong, resilient ligament with proper load-bearing capacity and long-term functionality	Weakened ECM; reduced stiffness; disorganized collagen fibrils; increased laxity	Weakened ligament structure; increased susceptibility to injury; pathological ECM remodeling	[42–44,114–116], [133–135]
Clinical & Therapeutic Implications	Rehabilitation protocols that apply “just right” and “optimal loading” mechanical stimulus; optimize ECM remodeling; maintain ligament phenotype	Need for interventions to restore anabolic balance; prevent ECM degradation; promote collagen synthesis	Therapeutic strategies to reduce inflammation; inhibit MMPs; manage oxidative stress; restore ECM integrity	[59–61], [133–135], [125–126]

Inadequate load (e.g., during immobilization) decreases integrin clustering and focal adhesion stability, reducing FAK phosphorylation and downstream signaling [125–126]. Without the requisite mechanical stimuli, ligament fibroblasts downregulate anabolic genes while upregulating catabolic mediators. These changes often manifest as an increase in collagen III at the expense of collagen I, disruption of fibril organization, and altered expression of tenascin-C, a glycoprotein that influences cell–matrix interactions and tissue repair processes [49–51, 127–129]. The result is a more disorganized ECM, reduced stiffness, and diminished load-bearing capability. Excessive mechanical loading, on the other hand, can induce cellular stress responses that involve the release of pro-inflammatory cytokines such as IL-1 $\beta$  and TNF- $\alpha$  [52–54, 130–132]. These cytokines stimulate pathways that enhance the synthesis and activation of matrix metalloproteinases (MMPs), enzymes that degrade collagen and other ECM components. As ECM integrity declines, the tissue becomes weaker and more susceptible to injury. Excessive loading may also alter the expression of key regulatory microRNAs that fine-tune collagen and MMP gene expression, reinforcing the breakdown of ECM and driving the tissue toward pathological remodeling [133–135].

In essence, the molecular adaptations of ligament cells to mechanical loading form a delicate continuum. At one end, physiologic loading maintains homeostasis by orchestrating a network of signaling molecules, transcription factors, and epigenetic regulators that promote collagen synthesis, appropriate proteoglycan levels, and stable cell phenotypes. At the other extreme, insufficient or excessive loading conditions lead to a downward spiral of altered integrin signaling, inflammatory mediator release, and enhanced ECM degradation.

## **Tendon-to-Ligament Transition. Anterior Cruciate Ligament Graft Remodeling**

A clinically relevant example of ligament cell plasticity at the molecular and cellular levels is demonstrated after anterior cruciate ligament (ACL) reconstruction with the use of tendon grafts. Common donor tissues, such as the hamstring (semitendinosus/gracilis tendon graft) or patellar tendon, initially retain their inherent tendon-like molecular profile, characterized by a highly aligned, predominantly type I collagen matrix and minimal elastin or proteoglycan content [55–57, 136–138]. Placed into the knee joint, these tendon grafts are suddenly exposed to a complex mechanical milieu fundamentally different from the unidirectional tensile environment of a tendon's native placement.

Over time, the graft undergoes a process termed “ligamentization,” in which mechanical loading patterns, local biochemical signals, and the joint's synovial environment collaborate to drive a molecular and cellular transformation from a tendon-like to a ligament-like phenotype [58–61, 139–140]. This biological adaptation involves complex remodeling, including cellular infiltration, vascularization, extracellular matrix reorganization, and collagen maturation. Ligamentization occurs in distinct phases, beginning with early necrosis, followed by revascularization, cellular repopulation, and progressive structural integration into the knee joint. The tendon graft healing process in a bone tunnel has been studied in animal models [141]. Initially, the graft-tunnel interface fills with vascular granulation tissue rich in type III collagen. Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) stimulate macrophage influx and fibroblast enlargement. Chondroid cells deposit type II collagen, supporting new bone formation [141]. By 3–4 weeks, the matrix matures into Sharpey-like collagen fibers bridging the bone and graft. These fibers, composed of type III collagen, integrate into surrounding bone to resist shear stress, with their size correlating to graft pull-out strength. The process takes 8–30 weeks [141], with graft attachment strengthening as bone grows into the interface tissue [142].

At the heart of this transformation are changes in mechanotransduction pathways: integrins bind to newly abundant ECM components, including type III collagen, elastin, decorin, and biglycan. These integrin-mediated adhesions remodel focal adhesions and alter the actin cytoskeleton organization, shifting intracellular tension and triggering new signaling cascades [62–64, 143–144]. On the molecular level, this transition is orchestrated by shifts in gene expression that reflect the ligament's need to accommodate more complex, multidirectional loads. Transcription factors such as Scx and Mxk may adjust their relative expression patterns in response to altered mechanical stress and growth factor gradients [145–147]. Additionally, extracellular signals from TGF- $\beta$ , FGF, and VEGF support ECM remodeling, neo-vascularization, and cellular proliferation [148–150]. TGF- $\beta$ , for instance, drives the production of type III collagen and elastin, while VEGF promotes angiogenesis, ensuring that the evolving graft is well-vascularized and metabolically supported. FGFs and CTGF further influence collagen fibrillogenesis and crosslinking, reinforcing the emerging ligamentous structure. Integrin signaling feeds into MAPK and PI3K/AKT pathways, modulating the balance of anabolic and catabolic genes. As these pathways are tweaked by the new mechanical environment, cells increase the synthesis of small leucine-rich proteoglycans (SLRPs) like decorin and biglycan [151–153]. These SLRPs guide collagen fibrillogenesis, adjusting fibril diameter distributions and introducing a characteristic crimp pattern more akin to ligament tissue. Over time, the mechanical properties evolve: the graft's collagen fibrils become less linear and more varied in diameter and crimp,

imparting increased compliance and an ability to handle shear and rotational forces typical of ligament loading [65–67, 154–156]. At the cellular level, epigenetic modifications (e.g., changes in histone acetylation, DNA methylation, and microRNA profiles) may also play a role in stabilizing the ligament-like transcriptional program [157–159]. These epigenetic changes help “lock in” the new molecular identity, ensuring that the once tendon-specific fibroblasts now consistently express genes associated with ligament biology and maintain a ligamentous ECM architecture.

Biologic augmentation strategies, such as delivering specific growth factors (e.g., TGF- $\beta$  or VEGF), using gene editing techniques to upregulate ligament-associated transcription factors, or modulating epigenetic regulators, can further hasten the tendon-to-ligament transition [160–165].

## Regulatory Networks and Transcriptional Control

Ligament cell fate and ECM composition are governed by an intricate regulatory landscape that integrates mechanical cues with transcriptional and epigenetic mechanisms. Central to this network are key transcription factors (TFs) that respond to the tissue's mechanical environment and orchestrate the gene expression patterns needed for proper ligament structure and function. Scx, a basic helix-loop-helix transcription factor, is one of the most extensively studied regulators of tendon and ligament development. Scx directs the transcription of collagen genes (e.g., COL1A1, COL3A1) and other ECM components, ensuring the formation of robust, load-bearing collagen fibrils [166–168]. By interacting with cofactors and binding to enhancer regions of ECM-related genes, Scx helps maintain the ligament's tensile strength and prevents the adoption of a non-ligamentous phenotype. Similarly, Mxx, a homeodomain transcription factor, plays critical roles in specifying tendon/ligament lineage commitment and sustaining ligament cell identity [169–170]. Mxx influences collagen fibrillogenesis and ECM organization, complementing the actions of Scx and ensuring that mechanical signals translate into a stable gene expression program. Another important transcription factor, EGR1, modulates the tissue's response to injury and mechanical stress [171]. EGR1 is rapidly induced by extracellular signals and biomechanical loading, influencing genes involved in ECM remodeling, growth factor signaling, and cytoskeletal architecture. By adjusting the levels of EGR1, ligament cells can tune their repair strategies—upregulating MMPs and proteoglycans as needed during healing and downregulating them once homeostasis is restored. In this manner, EGR1 acts as a molecular rheostat that balances anabolic and catabolic processes in response to changing mechanical environments.

These transcription factors do not act in isolation. Instead, they function within larger regulatory networks where mechanical signals—transduced via integrins, focal adhesion complexes, and the cytoskeleton—converge on the cell nucleus to modulate TF activity [172–173]. Mechanical stretch or compression can alter TF nuclear localization, protein stability, and DNA-binding affinity. Certain mechanosensitive co-activators, such as YAP/TAZ, may also participate in this regulatory matrix [174–175]. YAP/TAZ activity is influenced by cytoskeletal tension and focal adhesion assembly, allowing cells to integrate mechanical information with biochemical cues and adjust gene expression patterns accordingly. In addition to these TF-driven regulatory circuits, epigenetic mechanisms provide another layer of fine-tuning. DNA methylation and histone modifications (e.g., acetylation, methylation) can dynamically alter chromatin structure, influencing the accessibility of transcription factor binding sites and thereby regulating the transcriptional output in ligament fibroblasts [176–178]. Mechanical cues have been shown to modulate chromatin organization, potentially through mechano-sensitive chromatin remodeling complexes that determine which genes are actively transcribed or repressed in response to load [179–180].

MicroRNAs (miRNAs) are emerging as critical post-transcriptional regulators of gene expression in ligament biology. These small, non-coding RNAs bind to target mRNAs and either repress their translation or induce their degradation. For instance, miRNAs can

finely adjust collagen and MMP expression, ensuring that ECM synthesis and degradation remain in balance [181–183]. By selectively modulating levels of key structural proteins and enzymes, miRNAs help maintain tissue integrity and responsiveness to mechanical stimuli. Changes in miRNA expression profiles after injury or altered loading conditions can tilt the balance between anabolic and catabolic activities, contributing to either successful healing or degenerative changes. For example, delivering small molecules or gene-editing reagents (e.g., CRISPR/Cas9) could modulate the activity of *Scx*, *Mxk*, or *EGR1*, accelerating the tendon-to-ligament transition in ACL grafts [184–186]. Likewise, manipulating miRNA expression or histone-modifying enzymes could prevent unwanted fibrocartilaginous transformations, maintain ECM homeostasis, and promote superior mechanical function [187–188].

In essence, the molecular and cellular regulatory networks controlling ligament cell fate and ECM composition form an adaptive system that continually integrates mechanical and biochemical signals.

## Growth Factors, Cytokines, and Inflammatory Mediators

Beyond structural proteins, the mechanical environment of the ligament coordinates a multifaceted molecular dialogue involving growth factors, cytokines, and other signaling molecules that guide ligament cell fate and ECM organization. Ligament fibroblasts—attuned to subtle changes in tissue tension and stiffness—respond to appropriate tensile strain by adjusting their secretome and receptor expression patterns [189–191]. When integrins at the cell surface detect physiological loading, they cluster within focal adhesions and engage intracellular adaptors to activate downstream signaling pathways [192–194]. This mechanotransduction process triggers the upregulation and release of key growth factors, such as TGF- $\beta$  isoforms, which translocate to the nucleus via SMAD-dependent pathways. There, they influence chromatin structure and transcription factor binding, increasing the expression of collagen (particularly types I and III) and proteoglycans (such as decorin and biglycan). These ECM components enhance the tissue's tensile strength, maintain proper fibril spacing, and preserve its viscoelastic properties [76, 195–197]. Similarly, FGF and IGF-1 (Insulin-like Growth Factor 1) operate as potent modulators of cellular proliferation and ECM turnover by engaging their receptor tyrosine kinases and activating MAPK and PI3K/AKT cascades [198–200]. At the molecular level, these pathways converge on transcriptional regulators that orchestrate gene programs for ECM synthesis, cytoskeletal remodeling, and metabolic adaptations. FGF-driven signals, for instance, can fine-tune actin filament organization, influencing the spatial arrangement of collagen fibrils. IGF-1, in turn, can bolster anabolic metabolism, ensuring that fibroblasts have sufficient energy and substrates for ECM protein synthesis [201–203]. CTGF, another crucial factor, integrates mechanical cues and growth factor signals by localizing to focal adhesions and influencing collagen fiber alignment. CTGF's presence refines fibril architecture, ensuring that newly deposited collagen fibers form robust, mechanically optimized networks [77–79, 204–206]. On a molecular scale, these growth factors modulate the balance between collagen crosslinking enzymes (e.g., lysyl oxidase) and proteolytic enzymes, guiding fibril maturation and stiffness. However, this delicately balanced molecular system can be perturbed by injury, abnormal loading patterns, or local hypoxia [207–209]. Excessive mechanical strain or reduced loading leads to alterations in integrin-mediated signaling, causing shifts in the fibroblast secretome. Under these conditions, pro-inflammatory cytokines such as IL-1 $\beta$  and TNF- $\alpha$  surge [210–212]. At the cellular level, these cytokines activate NF- $\kappa$ B and related transcriptional networks, upregulating MMPs and aggrecanases that degrade ECM components and weaken the overall scaffold [80–82, 213–215]. High levels of NO (Nitric Oxide) further compound these issues by disrupting growth factor receptor function and interfering with proper ECM protein folding and crosslinking [216–218]. NO's reactive intermediates can modify the redox state of critical signaling proteins, diminishing the efficiency of growth factor-driven anabolic pathways and exacerbating collagen breakdown. This molecular environment fosters a feedback loop where degraded ECM fragments and persistent inflammatory signals

perpetuate a cycle of tissue degeneration, scar formation, and biomechanical insufficiency.

From a cellular perspective, fibroblasts exposed to these pathological cues alter their gene expression profiles, increasing expression of stress-responsive genes and proteolytic enzymes while reducing collagen synthesis [219–221]. Changes in actin cytoskeleton organization and nuclear shape reflect shifts in transcription factor localization and chromatin accessibility, ultimately skewing the cell toward a catabolic phenotype [222–224]. Over time, these molecular changes reshape the local microenvironment, favoring fibrocartilaginous or scar-like tissue rather than a functional ligament structure. To reverse or prevent these maladaptive processes, interventions that target molecular pathways hold promise [225–227]. Such precise mechanical stimulation preserves TGF- $\beta$ , FGF, IGF-1, and CTGF's anabolic effects on collagen alignment and ECM deposition while dampening pro-inflammatory signals. Pharmacological agents or biologics that block IL-1 $\beta$  or TNF- $\alpha$  receptors, neutralize NO, or inhibit MMP activity can restore a more regenerative molecular environment. Gene editing tools or miRNA modulators could be deployed to stabilize anabolic gene expression patterns or to rescue compromised integrin and growth factor signaling pathways [228–230].

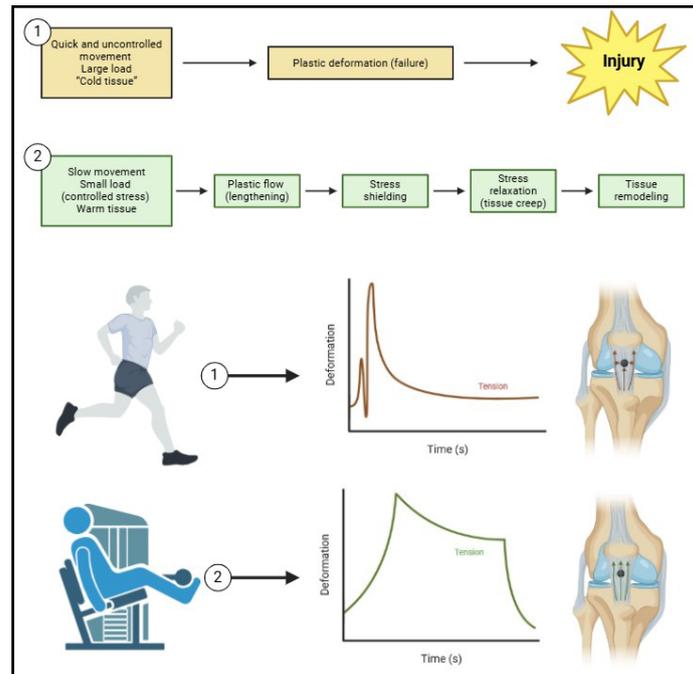
In summary, the molecular and cellular interplay between mechanical forces, growth factors, and inflammatory mediators is central to ligament homeostasis and repair. By understanding the precise molecular underpinnings—how integrin signaling interfaces with growth factor receptor activation, how cytokines distort transcriptional and epigenetic landscapes, and how NO modulates receptor sensitivity—clinicians and researchers can design strategies that maintain molecular homeostasis. This will improve ECM quality, ensure proper collagen fibrillogenesis, and ultimately lead to more resilient ligaments better able to withstand functional demands and recover from injury.

## Stress Shielding in Ligaments

The concept of stress shielding in ligaments emerges when portions of the tissue are effectively protected or “shielded” from the mechanical strains they would normally experience (Fig. 2). Under healthy conditions, ligaments and tendons rely on tension and mechanical loading cues to maintain their structural integrity, ensuring proper cell alignment, collagen fibrillogenesis, and tissue homeostasis. However, when stress is diverted or reduced—such as by the presence of an adjacent stiffer material, a parallel healthy tissue segment, or changes in tissue composition—ligament cells are deprived of the mechanical signals needed to sustain normal ECM organization and tenogenic (ligament-like) gene expression [231–233]. Ligaments exhibit viscoelastic properties characterized by creep, hysteresis, and stress-relaxation—the phenomenon where tissue stress decreases over time under a constant strain. Normally, small, cyclic load-unload patterns and brief stress-relaxation intervals help maintain ligament health, allowing collagen fibrils to reorganize, water and solute to redistribute, and cells to recover metabolically. When stress is permanently shielded from certain ligament regions, those areas experience drastically reduced load and, thus, diminished opportunities for periodic stress-relaxation. Without adequate stress-relaxation cycles, fibroblasts fail to engage in the micro-adjustments (e.g., cytoskeletal remodeling, local collagen reorientation) vital for maintaining or restoring tissue tensile properties.

In healing or injured ligament tissues, the formation of a compliant, disorganized scar in parallel with stiffer, healthier tissue leads to a significant mismatch in mechanical properties [234–235]. Because the intact portion of the ligament (or an external stiff structure, like a surgical implant) bears most of the load, the more compliant scar region undergoes relatively less tension. This reduction in stress transmission to the scar is known as stress shielding. The under-loaded scar tissue experiences minimal stress-relaxation episodes, limiting its ability to reorganize collagen fibrils. Ligament fibroblasts in the shielded region lose crucial mechanical cues, reduce matrix turnover, and fail to form well-aligned collagen fascicles capable of bearing load [236–238]. Without adequate tensile loading, cells within the scar

**Fig. 2.** This Fig. compares two loading scenarios—(1) quick, uncontrolled movement with large loads on “cold” tissues versus (2) slow, controlled movement with smaller loads on “warm” tissues—and illustrates how stress shielding and stress-relaxation can either contribute to injury or promote tissue remodeling. In the top panel (1), a sudden high load induces plastic deformation (failure) in the ligament, potentially leading to injury. Because the tissue is not given time to accommodate or reorganize collagen fibrils, the deformation overshoots the ligament’s elastic capacity, resulting in permanent damage. By contrast, the bottom panel (2) shows a gradual, lower-intensity load that proceeds through phases of plastic flow, stress shielding, stress relaxation, and ultimately tissue remodeling. Here,



the tissue benefits from slow loading (e.g., warm-up exercises or controlled rehabilitation protocols) that facilitate creep, water redistribution, and cytoskeletal adjustments. Although stress shielding can still occur if certain regions of the ligament bear less load than others, timely stress-relaxation intervals allow fibroblasts to reorient collagen fibrils and maintain tenogenic gene expression. This is critical for avoiding disorganized, scar-like tissue and instead promoting healthier ECM architecture over time. The time–deformation graphs depict how, under quick heavy loads, tissue stress initially spikes and may quickly exceed its failure threshold. Under slower, methodical loading, tension climbs more gradually and then partially relaxes, allowing collagen realignment and micro-adjustments that protect ligament integrity. The knee joint schematic on the right underscores how these loading patterns impact ligament homeostasis: uncontrolled movement risks plastic deformation and injury, whereas controlled stress cycles combined with adequate rest and stress-relaxation intervals encourage adaptive remodeling and strengthen the ligament’s load-bearing capacity. High intensity activities monitoring is crucial in injury prevention.

cannot properly align; consequently, collagen fibrils remain disordered and fail to mature into a load-bearing, ligament-like matrix. Over time, diminished stress-relaxation intervals can further hasten the fibrocartilaginous transition of the tissue, making it more vulnerable to mechanical failure.

Molecularly, in the absence of sufficient tensile stress and normal stress-relaxation cycles, transcription factors such as Mohawk (Mkx) and Egr1 remain low, while chondrogenic markers like Sox9 and Col2a1 rise [239–241]. In a properly stressed ligament, periodic strain (and associated stress-relaxation) triggers integrin–FAK–MAPK signaling that drives collagen type I expression, organizes fibrils, and promotes the tenogenic phenotype. By contrast, shielded regions fail to sustain these signals. Elevated Sox9, collagen type II, and proteoglycan content emerges, resembling tissue more akin to cartilage than ligament. Scar tissue retains immature collagen crosslinks and smaller fibril diameters, lacking the robust load-bearing capacity typical of healthy ligament [242–244]. Experimental evidence supports the role of stress shielding in this detrimental cascade. Insertion of a stiffer material in parallel with a healthy tendon or ligament decreases the tensile forces experienced by the native tissue, leading to decreased load-bearing capacity, collagen disorganization, and hypercellularity—changes characteristic of scar formation [245–247]. These shielded regions also exhibit insufficient stress-relaxation behavior, indicating minimal opportunities for reorienting collagen fibrils and remodeling the matrix.

Conversely, lowering the stiffness of the healthy portion of the tissue or adjusting loading protocols (such as introducing isometric tension that facilitates creep and thus better load transfer to scarred areas) can reintroduce mechanical signals to shielded cells [248–250]. These signals restore partial stress-relaxation cycles and help fibroblasts regain a tenogenic phenotype. Elevated *Mkx/Egr1* expression, improved collagen alignment, and more mature crosslinking patterns are often observed when mechanical cues are reestablished.

In summary, stress shielding in ligaments is a mechanically driven phenomenon wherein portions of a healing or regenerating ligament experience insufficient tensile loading and inadequate stress-relaxation intervals. This lack of mechanical input prevents proper ECM maturation and gene expression patterns essential for restoring healthy ligament structure and function, thereby contributing to the persistence of disorganized, scar-like tissue rather than promoting regenerative healing. Reintroducing tension—via modifications in loading protocols, stiffness gradients, or physical rehabilitation regimens that encourage periodic stress-relaxation—may potentially restore normal collagen architecture and bolster the mechanical resilience of the ligament over time.

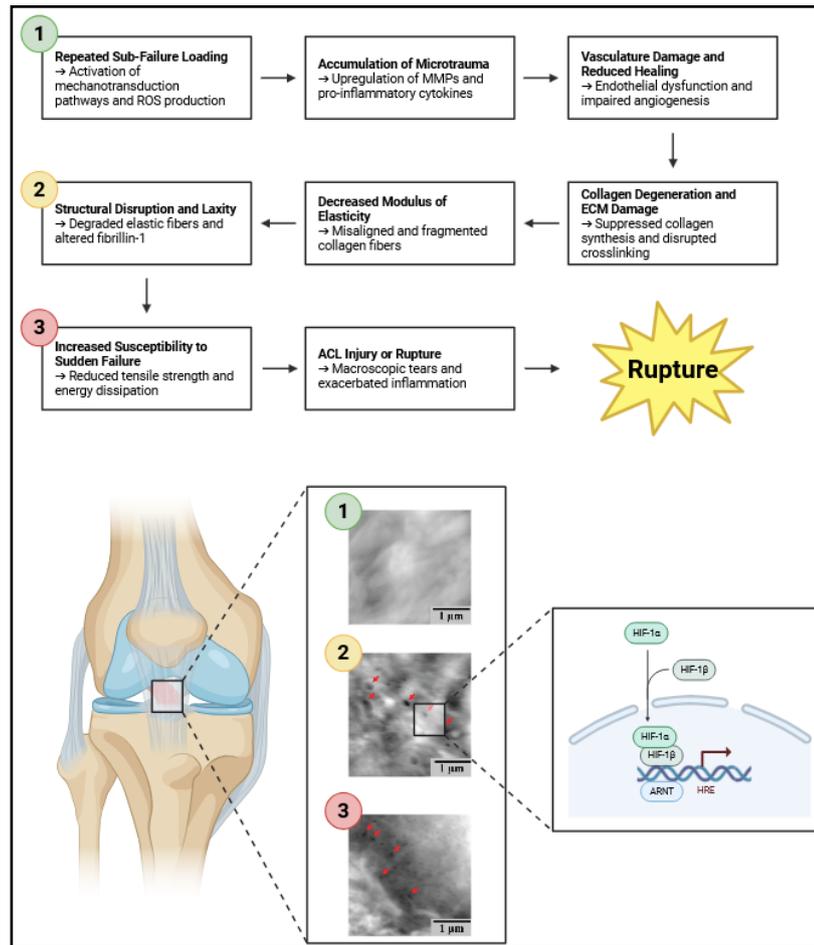
### ACL Microtrauma and Overuse

Microtrauma accumulates at the molecular and cellular levels as the ACL is subjected to repetitive, sub-failure loads that exceed its capacity for timely repair. At the core of this process is the ligament's resident fibroblasts, which continuously sense and respond to mechanical signals through integrin-mediated focal adhesions, the cytoskeleton, and associated mechanotransduction pathways [251–253]. Under healthy loading conditions, these cells maintain the ECM by synthesizing collagen and other proteins, adjusting fibril diameter and organization, and balancing anabolic and catabolic activities. However, when microtrauma occurs faster than the tissue can repair, molecular homeostasis is disrupted (Fig. 3).

Molecularly, repetitive sub-failure strains can cause partial unfolding of collagen's triple-helical structure at the fibrillar and sub-fibrillar levels. This mechanical deformation exposes cryptic binding sites that increase susceptibility to enzymatic cleavage by MMPs and aggrecanases [254–256]. The altered mechanical environment shifts fibroblast gene expression patterns, downregulating genes associated with stable collagen assembly and upregulating those involved in ECM remodeling and degradation. Over time, these changes compromise the uniform alignment and crosslinking of collagen fibrils. As collagen fibrils become disorganized or partially denatured, the mechanical stiffness and tensile strength of the ligament decrease, lengthening the force-displacement “toe region” of the stress-strain curve and increasing ligament laxity [257–259].

At the cellular level, the reduced ECM integrity and altered load distribution modify integrin engagement, focal adhesion assembly, and downstream signaling via MAPKs, PI3K/AKT, and Rho GTPases [260–262]. Fibroblasts exposed to these altered mechanical cues may produce more inflammatory mediators and shift toward a more catabolic phenotype, increasing MMP secretion and decreasing collagen synthesis. In addition, vasculature damage from microtrauma diminishes nutrient and oxygen delivery, inducing localized hypoxia [263–265]. Ligament cells, possessing relatively low baseline metabolic activity, are generally resistant to short-term hypoxia. However, chronic or repeated episodes can hamper cellular respiration, reduce ATP production, and limit the resources needed for collagen synthesis and proper fibril maturation. The resulting ischemic environment may trigger oxidative stress and increase reactive oxygen species (ROS) production, further impacting protein folding, crosslink integrity, and cell membrane stability [266–268]. Cellular responses to microtrauma also involve subtle changes in mechanosensitive ion channels, growth factor receptor signaling, and epigenetic regulation [269–271]. For instance, changes in loading frequency or magnitude can alter the expression of transcription factors like *Scx* and *Mkx*, as well as microRNAs controlling collagen deposition and MMP activity. Poor vascularity within certain regions of the ACL, especially at the entheses, slows the infiltration of progenitor cells

**Fig. 3.** Presents a detailed schematic divided into eight interconnected panels, each representing a distinct stage in the progression from repeated sub-failure loading to ACL injury or rupture. The sequence begins on the left with Repeated Sub-Failure Loading and culminates on the right with ACL Injury or Rupture. Each panel not only depicts the mechanical and structural alterations within the ligament but also delves into the underlying molecular responses that drive these changes. The progression commences with Repeated Sub-Failure Loading, where athletes engage in repetitive movements such as jumping, cutting, and pivoting. These activities subject the ACL to cyclic tensile and shear forces that are insufficient to cause immediate structural failure but are significant enough to activate mechanotransduction pathways within ACL fibroblasts. This mechanical stress triggers the activation of transcription factors NF- $\kappa$ B (Nuclear Factor kappa-light-chain-enhancer of activated B cells) and AP-1 (Activator Protein 1), which translocate to the nucleus to initiate the expression of various inflammatory genes. Concurrently, the production of Reactive Oxygen Species (ROS) increases within ligament cells, symbolizing the onset of oxidative stress. As loading continues, Accumulation of Microtrauma occurs, evidenced by minor, reversible damage to the extracellular matrix (ECM), particularly the collagen fibers. At the molecular level, there is an elevation in Matrix Metalloproteinases (MMP-1 and MMP-13), enzymes responsible for degrading collagen and other ECM proteins. The synthesis of pro-inflammatory cytokines such as Interleukin-1 beta (IL-1 $\beta$ ) and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) is upregulated, enhancing MMP activity while simultaneously suppressing ECM synthesis. Additionally, alterations in integrin signaling pathways disrupt the critical communication between cells and the ECM, further exacerbating tissue degradation. Progressing to Vasculature Damage & Reduced Healing, the persistent microtrauma inflicts damage on the microvasculature within the ACL, leading to reduced blood flow. This hypoxic environment stabilizes Hypoxia-Inducible Factor 1-alpha (HIF-1 $\alpha$ ), which alters gene expression to adapt to low oxygen conditions. However, chronic stabilization of HIF-1 $\alpha$  results in maladaptive responses, including the downregulation of Vascular Endothelial Growth Factor (VEGF), thereby inhibiting angiogenesis. Concurrently, apoptotic pathways are activated in ACL fibroblasts through the involvement of caspases and the regulation of BAX/Bcl-2 proteins, leading to programmed cell death and further diminishing the ligament's capacity for repair and regeneration. The subsequent stage, Collagen Degeneration & ECM Damage, is marked by diminished and disorganized collagen fibers within the ligament. There is a suppression of type I collagen genes (COL1A1 and COL1A2) and a downregulation of lysyl oxidase, an enzyme crucial for collagen crosslinking. This weakening of collagen crosslinks undermines the structural integrity of the collagen fibers. Additionally, aggrecanases degrade glycosaminoglycans (GAGs), essential components that maintain the ECM's viscoelastic properties. The degradation of GAGs further compromises the ligament's structural resilience, making it more susceptible to mechanical deformation. Moving to Decreased Modulus of Elasticity, the ligament exhibits reduced stiffness and increased flexibility. This change is a direct consequence of misaligned and fragmented collagen fibers, which disrupt the uniform distribution of mechanical stress across the ligament. Oxidative modifications, such as carbonylation, impair the mechanical properties of collagen, while a reduced density of intact collagen fibrils diminishes the ligament's elastic modulus. These molecular alterations weaken the ligament's ability to absorb and distribute tensile forces effectively. In the stage of Structural Disruption & Laxity, the ligament appears stretched and less taut, indicating increased laxity. Elastin fibers undergo enzymatic degradation, and the expression of tenascin-C, a glycoprotein involved in tissue remodeling, is upregulated. These changes contribute to the disorganization of the ECM, further compromising the ligament's structural integrity. The loss of fibril alignment adversely affects load distribution, rendering the ligament more prone to deformation and injury during normal activities. As damage accumulates, the ligament reaches a state of Increased Susceptibility to Sudden Failure. The overall structure of the ACL is significantly weakened, with visible areas of potential rupture. Cellular viability decreases due to ongoing apoptosis and necrosis of ACL fibroblasts, impairing the ligament's ability to maintain and repair the ECM. Disruptions in integrin and growth factor signaling hinder the ligament's adaptive responses to mechanical stress, while protein misfolding and aggregation further compromise cellular functions. These molecular dysfunctions collectively reduce the ligament's resilience, making it highly susceptible to sudden failure. The final panel, ACL Injury or Rupture, depicts the complete tear of the ACL accompanied by surrounding inflammation. The collagen matrix can no longer withstand the tensile forces, resulting in macroscopic tears. An inflammatory cascade is activated, releasing damage-associated molecular patterns (DAMPs) that exacerbate tissue injury. The body's attempt to heal the damaged ligament involves scar tissue formation, characterized by disorganized fibroplasia that fails to restore the original function and integrity of the ligament. Fig. adapted from Chen J et. al., 2019).



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and reparative growth factors, prolonging the duration during which fibroblasts operate under suboptimal conditions [272–274]. This spatial heterogeneity in blood supply can create local “hotspots” where microtrauma accumulates, giving rise to region-specific ECM degeneration and eventual mechanical failure.

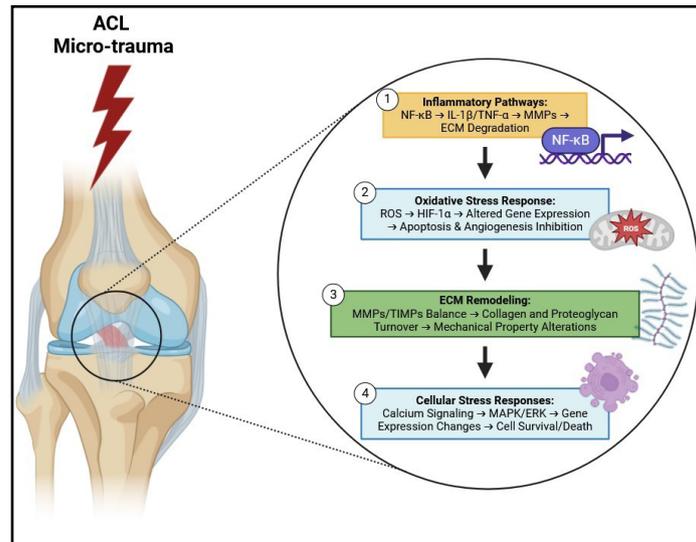
In addition, excessive exercise intensity or poor recovery strategies can chronically elevate ROS levels and pro-inflammatory cytokines in the ligament microenvironment (Fig. 4). This metabolic and oxidative imbalance places further strain on fibroblasts' repair capacity, reducing their ability to maintain a stable collagen network. Intermittent exercise, with adequate rest and nutrient support, can improve redox homeostasis and antioxidant defenses, enabling fibroblasts to better manage oxidative stress and resume anabolic activities [275–277]. Appropriate timing of collagen peptide supplementation and balanced amino acid availability may support fibroblast function, enhancing collagen synthesis and improving the ECM's resilience to repeated loading [278–279]. Ultimately, microtrauma-induced changes at the molecular and cellular levels diminish the ligament's modulus of elasticity and predispose it to sudden fatigue failure under nominal loads. Repetitive sub-failure strain compromises the delicate equilibrium between ECM deposition and degradation, shifts fibroblast phenotypes, and disrupts nutrient and oxygen delivery, collectively weakening the ACL's structural integrity [280–282]. By optimizing cellular metabolism, redox homeostasis, and growth factor signaling, it may be possible to enhance the ACL's capacity for microtrauma repair, thereby extending its functional endpoint and reducing the risk of catastrophic rupture. In cases where surgical intervention is not immediately required or contraindicated, conservative treatment strategies play a crucial role in managing ACL injuries. Structured rehabilitation programs focusing on neuromuscular training, proprioception enhancement, and quadriceps-hamstring coactivation can aid in stabilizing the knee joint and compensating for ligamentous insufficiency. Additionally, interventions such as bracing, controlled progressive loading, and biomechanical gait retraining help mitigate excessive stress on the ACL while promoting adaptive tissue remodeling.

## Ligament Chronobiology: Loading and Unloading

Ligaments exist in a dynamic mechanical environment where the interplay of loading and unloading cycles orchestrates their molecular and cellular behavior. Chronobiology—the time-dependent patterning of biological processes—provides a framework for understanding how the frequency, duration, and intensity of mechanical stimuli modulate the ECM composition, collagen synthesis, and cellular phenotype of ligament fibroblasts over periods of strain and rest [283–285]. Rather than a static tissue, the ligament responds to mechanical cues in rhythmic fluctuations that shape gene expression, protein turnover, and cellular metabolism. At the molecular level, integrin-mediated mechanotransduction pathways continuously gauge tensile forces. During loading phases, integrin engagement with collagen and proteoglycans stabilizes focal adhesions, activating FAK and downstream MAPK (ERK, JNK, p38) and PI3K/AKT cascades [286–288]. This activation amplifies signals that promote collagen gene transcription (COL1A1, COL3A1), ECM deposition, and proper fibril alignment. Mechanosensitive ion channels, such as TRP and PIEZO channels, open under tensile or shear forces, allowing  $\text{Ca}^{2+}$  influx [289–291]. Elevated intracellular  $\text{Ca}^{2+}$  fine-tunes cytoskeletal organization and enhances transcription factor activity, reinforcing ECM assembly. Growth factors like TGF- $\beta$ , FGF, and CTGF integrate into these pathways, leveraging mechanical cues to boost collagen crosslinking, fibrillogenesis, and mechanical strength [292–294].

Conversely, unloading intervals modulate these molecular networks differently. Without sufficient mechanical stimulation, integrin clustering diminishes, lowering FAK activity and reducing anabolic signaling [295–297]. Closed mechanosensitive ion channels limit  $\text{Ca}^{2+}$  entry, diminishing transcription of ECM proteins and potentially upregulating catabolic mediators such as MMPs. With persistent unloading, fibroblasts may shift their gene expression patterns toward more fibrocartilaginous characteristics, reducing collagen

**Fig. 4.** Presents a multi-layered schematic that delineates the intricate molecular mechanisms driving the progression from repeated sub-failure loading to ACL injury or rupture. The illustration is organized into four primary interconnected pathways—Inflammatory Pathways, Oxidative Stress Response, Extracellular Matrix (ECM) Remodeling, and Cellular Stress Responses—each highlighting a critical aspect of the cellular and molecular responses to mechanical stress within the ACL. These pathways collectively demonstrate the complex interplay between inflammatory signaling, oxidative stress, ECM degradation, and cellular stress responses, culminating in the structural failure of the ligament. The progression begins with repeated sub-failure mechanical loading, where cyclic tensile and shear forces are applied to ACL fibroblasts without causing immediate structural failure. This mechanical stress activates the NF- $\kappa$ B (Nuclear Factor kappa-light-chain-enhancer of activated B cells) signaling pathway through mechanotransduction mechanisms. Once activated, NF- $\kappa$ B translocates



to the nucleus, initiating the expression of various inflammatory genes. This upregulation leads to the synthesis of pro-inflammatory cytokines such as Interleukin-1 beta (IL-1 $\beta$ ) and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), which amplify the inflammatory response within the ligament tissue. These cytokines further stimulate the expression and activation of Matrix Metalloproteinases (MMPs), including MMP-1 and MMP-13, enzymes responsible for degrading key components of the ECM, particularly type I collagen. The heightened activity of MMPs disrupts the integrity of the ECM, thereby compromising the ACL's ability to withstand tensile forces and contributing to ligament laxity and vulnerability to injury. Simultaneously, sub-failure mechanical loading elevates the production of Reactive Oxygen Species (ROS) within ACL fibroblasts, inducing oxidative stress. Elevated ROS levels, coupled with resulting hypoxic conditions, stabilize Hypoxia-Inducible Factor 1-alpha (HIF-1 $\alpha$ ) by preventing its degradation. Stabilized HIF-1 $\alpha$  translocates to the nucleus and alters gene expression to adapt to low oxygen environments. However, chronic stabilization of HIF-1 $\alpha$  leads to maladaptive responses, including the induction of apoptotic signaling pathways involving caspases and the regulation of BAX/Bcl-2 proteins, culminating in programmed cell death of ACL fibroblasts. Additionally, chronic inflammation and HIF-1 $\alpha$ -mediated gene expression downregulate Vascular Endothelial Growth Factor (VEGF), impairing the formation of new blood vessels and further reducing the ACL's capacity for repair and regeneration. In the realm of Extracellular Matrix (ECM) Remodeling, the balance between Matrix Metalloproteinases (MMPs) and their natural inhibitors, Tissue Inhibitors of Metalloproteinases (TIMPs), is crucial. Chronic mechanical stress disrupts this balance by upregulating MMPs while downregulating TIMPs, tipping the scale toward ECM degradation. Enhanced MMP activity leads to increased breakdown of type I collagen and proteoglycans such as decorin and biglycan, which are essential for maintaining the viscoelastic properties of the ECM. The degradation of these components results in reduced tensile strength and elasticity of the ACL, making the ligament more susceptible to deformation and injury under normal physiological loads. Concurrently, Cellular Stress Responses are triggered by repetitive mechanical loading, which induces transient increases in intracellular calcium levels within ACL fibroblasts. Elevated calcium acts as a secondary messenger, initiating various intracellular signaling cascades, including the activation of the Mitogen-Activated Protein Kinase (MAPK) and Extracellular Signal-Regulated Kinase (ERK) pathways. These pathways regulate a wide array of cellular processes, including proliferation, differentiation, and apoptosis. Activation of MAPK/ERK leads to changes in the expression of genes involved in cell survival, proliferation, and ECM production. However, chronic activation under persistent mechanical stress can shift the balance toward catabolic gene expression profiles, resulting in decreased cellular viability. Depending on the context and duration of signaling, ACL fibroblasts may undergo apoptosis or necrosis, thereby impairing the ligament's capacity to maintain and repair the ECM. The integration of these four pathways—Inflammatory Pathways, Oxidative Stress Response, ECM Remodeling, and Cellular Stress Responses—creates a synergistic network that drives the degeneration of the ACL. Inflammatory signaling exacerbates ECM degradation by increasing MMP activity, while oxidative stress impairs vascular health and promotes apoptosis of essential fibroblasts. The imbalance in ECM remodeling further weakens the ligament's structural integrity, and the cellular stress responses reduce the ligament's ability to repair and regenerate damaged tissue. This cumulative effect of molecular and structural changes ultimately leads to the rupture of the ACL, especially when the ligament is subjected to additional mechanical loads that exceed its compromised capacity.

tensile strength and increasing tissue vulnerability [298–300]. Epigenetic modifications (e.g., histone acetylation, DNA methylation) and altered growth factor signaling further entrench these changes, leading to disorganized collagen fibrils and weaker mechanical properties [301–303]. Ligament fibroblasts also exhibit circadian clock genes, including BMAL1 and CLOCK, which form the core transcriptional and translational feedback loops that drive rhythmic gene expression [283–285]. Mechanical stimuli can synchronize these circadian regulators, influencing downstream targets like PER and CRY, which in turn modulate ECM metabolism, MMP activity, and collagen homeostasis. During loading phases aligned with the circadian rhythm, BMAL1/CLOCK complexes may reinforce signals from FAK or Ca<sup>2+</sup>-dependent cascades, ensuring optimal collagen synthesis and repair. By contrast, misalignment—such as prolonged unloading or irregular mechanical stress—could disrupt the normal oscillations of BMAL1/CLOCK, attenuating the anabolic signals and predisposing the ligament to collagen disorganization or slow healing.

At the cellular level, ligament fibroblasts interpret loading cycles through cytoskeletal adjustments and changes in nuclear morphology. During repeated loading, the cytoskeleton aligns stress fibers, improves actin-myosin tension, and maintains nuclear shape favorable for activating pro-tenogenic transcription factors like Scx and Mxk [304–306]. These patterns ensure balanced collagen turnover and steady ECM renewal. Appropriate unloading intervals allow cells to restore ATP and nutrient levels, recover from oxidative stress, and recalibrate growth factor signaling [307–309]. However, excessive unloading intervals or irregular load withdrawal can induce a gradual loss of tenogenic phenotype, undermining tissue integrity and stiffness [310–312].

Incorporating periodic rest phases between loading cycles provides opportunities for fibroblasts to upregulate antioxidant defenses, maintain mitochondrial function, and ensure a sustainable balance between reactive ROS production and detoxification [313–315]. This cyclical nature of mechanical input and metabolic restoration exemplifies the principle that ligaments thrive under conditions where loading and recovery follow a coordinated temporal pattern—one that also interfaces with circadian regulators like BMAL1 and CLOCK. These circadian mechanisms may help schedule ECM synthesis and degrade misfolded proteins in sync with daily fluctuations in mechanical activity, promoting both structural integrity and metabolic efficiency.

These insights emphasize that ligament healing and adaptation are governed by cycles of mechanical stimulation rather than a simple progression of clock time. Restoration of tissue homeostasis, prevention of degenerative changes, and optimization of collagen alignment hinge on properly dosing mechanical inputs and adjusting rest periods to cellular and molecular demands. The chronobiological perspective suggests that merely counting days or weeks post-injury is insufficient for guiding rehabilitation protocols. Instead, the cumulative number of mechanical load cycles, their magnitude, frequency, and recovery intervals should dictate the progression of therapy [316–318]. Aligning these mechanical cycles with the circadian oscillations governed by BMAL1 and CLOCK may further refine the outcome, making therapies more effective in modulating ECM turnover and cellular metabolism.

Therefore, rehabilitation should not be measured merely by time but by cycles of mechanical loads. By calibrating loading cycles to the ligament's molecular and cellular rhythms—including circadian gene regulators—clinicians can better facilitate sustainable ECM remodeling, prevent maladaptive tissue responses, and ultimately enhance the durability and functionality of the healing ligament [319–321].

## Implications for Tissue Engineering and Regenerative Medicine

The deepening understanding of how ligament cells transduce mechanical signals into molecular and cellular adaptations is providing a robust framework for advanced tissue engineering strategies. In engineered ligament constructs, controlling the mechanical environment at the cellular and molecular levels is key. By using bioreactors that apply physiologically relevant tensile strains to cell-seeded scaffolds, researchers can modulate integrin clustering and focal adhesion formation, guiding cytoskeletal organization and downstream signal transduction [83–85, 322–324]. This mechanical stimulation ensures that cells express the correct repertoire of ECM genes—upregulating collagens I and III, elastin, and proteoglycans—and that newly deposited collagen fibrils become properly aligned, ultimately conferring the scaffold with ligament-like mechanical properties.

Further refinement of scaffold materials can elevate these efforts. Biomaterials that incorporate integrin-binding domains (e.g., RGD motifs) or mimic the native ECM composition provide the essential molecular cues required for proper cell adhesion and mechanosensing [86–88, 325–327]. By presenting ligand-binding sites that selectively engage integrins expressed by ligament fibroblasts, these scaffolds facilitate the formation of focal adhesions and enhance mechanotransduction efficiency. As a result, signaling pathways such as MAPK (ERK, JNK, p38) and PI3K/AKT can be activated more precisely, fine-tuning gene expression

and ensuring that the developing construct attains the appropriate density, stiffness, and viscoelastic properties characteristic of native ligaments.

Equally important is the biochemical milieu. Delivering growth factors or cytokines—such as TGF- $\beta$ , FGF, PDGF, and VEGF—in a controlled manner can further stimulate ECM synthesis, improve collagen fibril organization, and support neovascularization [89–92, 328–330]. Spatially and temporally controlled growth factor release can help synchronize cellular proliferation, differentiation, and matrix remodeling phases, thereby accelerating the ligamentization of tendon grafts or other engineered constructs. Such an approach mirrors *in vivo* regenerative processes, where growth factor gradients, mechanical load patterns, and cellular signals interact to shape tissue architecture and function [331].

Genetic and epigenetic interventions offer another frontier of possibility for guiding ligament cell fate at the molecular level. For instance, overexpressing Mxk, a transcription factor pivotal for tendon/ligament differentiation, can shift the transcriptome of progenitor cells or tendon-derived cells toward a ligamentous phenotype [93–95, 331–333]. Similarly, modulating microRNA (miRNA) profiles provides a means to fine-tune the balance between anabolic and catabolic processes. By controlling miRNAs that target collagen, MMPs, or growth factor signaling pathways, researchers can encourage stable ECM formation and prevent excessive matrix degradation or scar formation [334–336]. Such genetic or epigenetic “preconditioning” sets the stage for cells to respond optimally once they are placed under mechanical load in a bioreactor or implanted *in vivo*. With the right molecular switches flipped, the cells become more receptive to integrin-mediated signals, more efficient at organizing ECM, and better able to resist mechanical fatigue. Ultimately, these interventions strive to replicate the intricate molecular milieu and mechanical environment of native ligaments—where integrins, cytoskeletal tension, transcription factors, epigenetic marks, and growth factors interact seamlessly [337–339].

In animals studies the culmination of these efforts is the production of living ligament constructs that not only have the structural and mechanical integrity to replace damaged ligaments but also possess the molecular and cellular machinery to integrate with host tissues and adapt over time [340–342]. However, there is currently no scientific evidence available supporting this approach in humans.

## Tendon–Ligament Force Transmission and Weight Savings

Tendons and ligaments transmit tensile forces across skeletal joints, effectively redirecting the path of force transmission while allowing varying degrees of joint motion. Biomechanically, these tissues feature densely packed, parallel-aligned collagen fibers (primarily type I collagen), hierarchically organized into microfibrils, fibrils, and fascicles, interspersed with smaller amounts of elastin and proteoglycans. This hierarchical organization confers both strength and flexibility. Ligaments generally function to limit the range of joint movement and stabilize the joint via their passive viscoelastic properties [367–369], whereas tendons couple muscle contraction to bone movement, often spanning one or more joints. From a molecular standpoint, collagen forms the core of both tendon and ligament structure. Each type I collagen molecule is composed of three polypeptide chains (two  $\alpha 1$  chains and one  $\alpha 2$  chain) that form a triple helix. These helices self-assemble into collagen fibrils, which further aggregate into collagen fibers. Covalent crosslinks, often catalyzed by lysyl oxidase, greatly enhance tensile strength and optimize viscoelastic behavior. The density and nature of these crosslinks help tune creep, hysteresis, and resilience under cyclical load. Smaller molecules such as decorin, biglycan, and fibromodulin (proteoglycans) help regulate fibril diameter, spacing, and collagen fibrillogenesis. Meanwhile, glycoproteins (e.g., tenascin-C, fibronectin) mediate cell adhesion and matrix organization. These constituents also facilitate water retention and lubrication, contributing to energy dissipation (damping) and overall tissue viscoelasticity. Fibroblasts (in tendon) or ligament fibroblasts (sometimes termed “ligamentocytes”) reside along and within the collagen fascicles. Their integrin receptors bind ECM molecules, translating mechanical stimuli (e.g., stretch, load, shear)

into intracellular signals via mechanosensitive pathways such as focal adhesion kinase (FAK), mitogen-activated protein kinase (MAPK), and Rho/ROCK signaling. These pathways orchestrate collagen synthesis, ECM remodeling, and adaptive changes in matrix composition in response to altered loading regimes.

Such cell–matrix interactions enable tendons and ligaments to undergo homeostatic remodeling, maintaining a balance between matrix deposition and degradation (including matrix metalloproteinase activity). This balance is crucial for sustaining tissue integrity over millions of loading cycles. Under tensile loads, tendons and ligaments can achieve a tensile failure strength of approximately 100–140 MPa and an elastic modulus in the range of 1.0–1.5 GPa [370–373]. This combination of high strength and relatively low mass provides a significant advantage (i.e., “weight savings”) compared to muscle tissue, which is far denser and more metabolically active. Over a given length, one would require roughly 2,800 times more muscle mass to transmit the same force that a tendon (or ligament) can carry [374, 375]. Consequently, natural selection frequently favors long, slender tendons or ligaments in distal limb segments, especially when fine control is less important than efficient force conduction over distance. Tendon stiffness determines how much the muscle fibers must shorten to produce a given movement. Stiffer tendons transmit force with minimal elongation, whereas more compliant tendons can store and release elastic energy. During activities like running or hopping, this compliance can reduce muscle fiber length changes, thereby decreasing metabolic cost.

By contrast, ligaments generally have slightly lower stiffness and allow controlled elongation, limiting joint excursion while passively contributing to joint stability. They also reduce the need for active muscular stabilization in certain ranges of motion, thus saving metabolic energy.

Extreme examples of the ligamentous strategy appear in the distal limbs of horses and other ungulates (e.g., the digital suspensory ligament), where muscle fibers are vestigial or absent [374, 376–378]. These passive structures transmit force over long distances with minimal mass, significantly lowering energy costs required for posture and locomotion. Humans exhibit a less extreme but still notable reliance on tendons—particularly the Achilles tendon—for storing and releasing elastic energy during gait.

Because tendons and ligaments are metabolically inert in terms of direct ATP consumption, they reduce the overall cost of movement. During gait, tendons can store elastic energy when stretched and release it upon recoil, alleviating the need for muscle fibers to shorten extensively. This mechanism is central to the high efficiency observed in running animals and humans [379–381]. If a tendon is sufficiently compliant, the muscle attached can operate more isometrically, producing force at an optimal fiber length without large length changes. This reduces the metabolic cost of generating force compared to fibers undergoing significant contractions. Over many strides or steps, such energy savings accumulate, enabling greater endurance or speed. A trade-off arises between compliance for energy saving and the capacity for precise control over limb position. Longer, more compliant tendons can introduce delays or oscillations due to the elasticity in the system—particularly relevant when reflex delays (tens of milliseconds) are considered. However, a moderate level of tendon compliance can actually help buffer high-frequency perturbations and refine control of low-force tasks by absorbing sudden shocks [371, 382]. Consequently, muscle–tendon architectures vary across anatomical regions and species, reflecting different functional priorities (e.g., distal vs. proximal limb muscles).

## Stress-Strain Curve in Ligaments

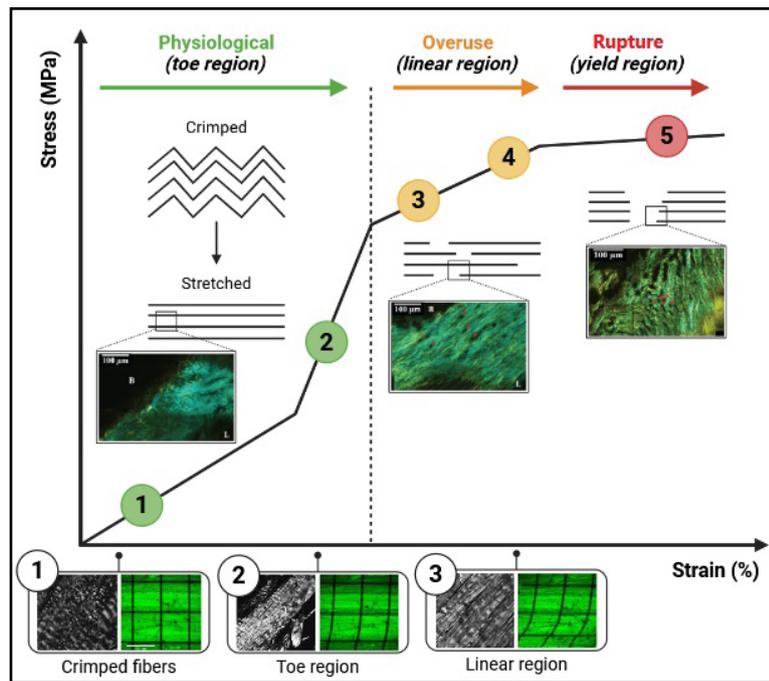
Ligaments exhibit a distinctive stress-strain curve that emerges from a sophisticated interplay of molecular components and cellular signaling pathways (Fig. 5). These mechanisms collectively enable ligaments to stabilize joints under various loads while allowing the flexibility required for normal movement [383]. Expanding on traditional views of collagen-dominated mechanics, recent insights reveal that ion channels, mechanosensing

molecules, and intricate molecular crosslinking processes are all essential for ligament health and function [384]. The primary load-bearing framework of ligaments is built on collagen, a protein that organizes into a triple-helical structure called tropocollagen [385]. Multiple tropocollagen molecules align into fibrils, which are further grouped into fibers. Ligament collagen is predominantly Type I, conferring high tensile strength, with smaller amounts of Type III contributing to tissue repair and structural support. A hallmark of collagen fibrils is their crimp pattern, a wave-like undulation that is straightened when tension is first applied. During the initial “toe” region of the stress-strain curve, this uncrimping accounts for a notable increase in strain with only a modest rise in stress. As loading continues, collagen fibrils become fully aligned and stiffen the tissue, creating the linear (elastic) region where stress increases sharply with strain [386]. Collagen’s mechanical integrity also relies on covalent crosslinks. Enzymatic crosslinks mediated by lysyl oxidase anchor collagen molecules together, fortifying fibrils against mechanical forces. Over time, non-enzymatic glycation (through advanced glycation end-products, or AGEs (Advanced Glycation End Products)) can further increase crosslink density [387]. This extra crosslinking often stiffens ligaments but reduces their extensibility, potentially heightening injury risk and limiting joint mobility, especially in aging tissues or in metabolic conditions such as diabetes [388].

Although collagen is the principal load-bearing component, proteoglycans—comprising a protein core decorated with GAG chains—play critical supporting roles [389]. These negatively charged GAGs attract water and cations, creating a swelling pressure that helps maintain fibril spacing and overall tissue hydration. Water, which makes up the majority of ligament mass, not only lubricates collagen fibrils but also facilitates nutrient transport and dissipates mechanical energy under repeated loading [390]. Glycoproteins like fibronectin and tenascin further organize fibrillar structures and connect cells (primarily fibroblasts) to the extracellular matrix. Although they contribute less to bulk tensile strength than collagen, they help mediate tissue remodeling and repair processes after microinjury [391]. Beyond the structural proteins, ligaments contain resident fibroblasts (sometimes referred to as ligament cells) that continuously monitor and respond to mechanical cues. Mechanosensitive ion channels embedded in fibroblast membranes act as molecular “switches,” transducing mechanical forces into electrochemical signals that guide gene expression and matrix remodeling. Certain TRP subfamily members (e.g., TRPV4) open in response to shear stress or stretch, allowing calcium ions to flow into cells [392]. Increased intracellular calcium can trigger a cascade of signaling events that influence collagen synthesis, proteoglycan turnover, and cytoskeletal reorganization. Piezo1 and Piezo2 are large mechanosensitive ion channels that open upon membrane deformation. When ligaments are subjected to load, stretch-induced Piezo channel activation modulates the release of second messengers, leading to changes in collagen gene expression and cellular proliferation rates. Although not strictly ion channels, integrins—transmembrane receptors linking the extracellular matrix to the cytoskeleton—work in tandem with these channels. As integrins sense tension within collagen fibers, they help coordinate ion channel gating, generating complex feedback loops that ensure tissue homeostasis [393]. Through these ion channel pathways, fibroblasts can sense even small changes in the mechanical environment, adjusting matrix production and degradation to meet physiological demands. Excessive or insufficient mechanical stimulation can shift this balance, potentially weakening the ligament or promoting fibrotic changes [394].

Ligaments are viscoelastic: their response to load varies over time due to fluid movement and molecular rearrangements within the matrix [395]. Water and ions within the proteoglycan-rich network shift as load increases or decreases, allowing ligaments to exhibit phenomena such as creep (gradual elongation under a constant load) and stress relaxation (reduced stress under a constant strain). Collagen molecules can slide and reorient slightly when loaded, enabled by the glycoprotein and proteoglycan interfaces. Over repeated loading cycles, energy is lost (hysteresis) as collagen and matrix components continually adjust. The gating and inactivation rates of mechanosensitive ion channels also contribute to time-dependent behavior [396]. Rapid changes in tension may produce spike-like calcium influxes, while slower deformations can lead to more prolonged changes in channel activity and cell signaling. When load exceeds the ligament’s capacity, plastic

**Fig. 5.** Depicts the stress-strain curve of ligament tissue, highlighting the progressive molecular and structural changes that occur as ligaments are subjected to varying degrees of mechanical loading. The Fig. is segmented into five distinct stages, each representing a critical phase in the ligament's response to mechanical stress, ranging from appropriate loading that promotes tissue homeostasis to excessive strain leading to ligament rupture. At the onset, appropriate mechanical loading, characterized by cyclic tensile and shear forces below the threshold for immediate structural failure, activates the PIEZO1 signaling pathway within ligament fibroblasts. PIEZO1, a mechanosensitive ion channel, responds to mechanical stress by facilitating the influx of calcium ions ( $\text{Ca}^{2+}$ ) into the cells. This calcium influx



triggers downstream signaling cascades involving Calcium/Calmodulin-Dependent Kinases (CaMKs) and Protein Kinase C (PKC), which subsequently activate transcription factors such as CREB and YAP/TAZ. These transcription factors enhance the expression of genes responsible for extracellular matrix (ECM) synthesis, including type I collagen (COL1A1 and COL1A2), elastin, and proteoglycans like decorin and biglycan. The upregulation of these ECM components reinforces the ligament structure, improving its tensile strength and elasticity, thereby maintaining tissue homeostasis and preparing the ligament to better withstand future mechanical loads. As mechanical loading intensifies and strain exceeds the deviation point, the ligament's elastic behavior becomes disrupted. Excessive stress leads to the partial unfolding of collagen triple helices and the disruption of hydrogen bonds that stabilize collagen structures. This molecular unfolding reduces the tensile strength of collagen fibers, while proteoglycans undergo stretching, altering their interactions with collagen and compromising ECM organization. Concurrently, elevated mechanical stress activates the Mitogen-Activated Protein Kinase (MAPK) and Extracellular Signal-Regulated Kinase (ERK) pathways, as well as the NF- $\kappa$ B pathway, which upregulates pro-inflammatory cytokines such as IL-1 $\beta$  and TNF- $\alpha$ . These molecular changes initiate catabolic processes that tilt the balance towards ECM degradation and hinder anabolic repair mechanisms, marking the transition from elastic to plastic deformation of the ligament. Continuing to endure mechanical loading beyond the deviation point, collagen fibers attempt to compensate for the loss of load transfer mechanisms by sliding and stretching at the molecular level. This compensatory behavior generates shear forces that further disrupt the organized alignment of collagen fibrils. The increased shear stress induces the activation of Matrix Metalloproteinases (MMP-2 and MMP-9), which specifically target denatured collagen and gelatin, accelerating ECM degradation. Additionally, mitochondrial dysfunction induced by excessive mechanical stress elevates the production of Reactive Oxygen Species (ROS), contributing to oxidative damage of ECM proteins and cellular components. The combined effect of heightened MMP activity and ROS-mediated damage leads to the gradual degradation of collagen fibers, weakening the structural integrity of fiber bundles and setting the stage for potential rupture. As mechanical loading persists, the gradual degradation of collagen culminates in the rupture of fiber bundles. Sustained high levels of mechanical stress maintain elevated expression of MMP-1 and MMP-13, enzymes that actively degrade type I and type II collagen. Simultaneously, the expression of Tissue Inhibitors of Metalloproteinases (TIMPs) is reduced, diminishing their ability to counteract MMP activity and allowing unchecked ECM degradation. The oxidative environment and pro-inflammatory cytokines activate caspases and regulate BAX/Bcl-2 proteins, leading to programmed cell death (apoptosis) of ligament fibroblasts. The loss of viable fibroblasts impairs the ligament's capacity to synthesize and maintain ECM components, resulting in the fragmentation of the collagen matrix and the eventual rupture of fiber bundles under continued mechanical strain. Ultimately, accumulated damage from repetitive microstrains below the immediate damage threshold leads to the attainment of a critical damage point. At this juncture, the ligament's ability to remodel and repair the damaged matrix becomes severely compromised. Persistent strain disrupts collagen crosslinks and depletes glycosaminoglycans (GAGs), reducing ECM plasticity and limiting matrix flexibility. The apoptotic loss of fibroblasts and the induction of cellular senescence further diminish ECM synthesis and maintenance, preventing effective repair of microinjuries. Sustained pro-inflammatory cytokine release perpetuates a catabolic environment, exacerbating ECM degradation and inhibiting anabolic processes necessary for ligament healing. Once the critical damage threshold is surpassed, the ligament loses its capacity to undergo necessary plastic deformation, rendering it highly susceptible to sudden rupture even under normal physiological loads. This culmination of molecular and structural degradation underscores the importance of managing mechanical loading to prevent overload-related tendon and ligament disorders. Fig. adapter from Eisner LE et al., 2022).

(irreversible) deformation and ultimately tissue failure can ensue. Excessive stretching or repetitive loading can break the covalent bonds linking collagen molecules, leading to small-scale microtears. As more crosslinks fail, fibrils lose their aligned structure and the matrix architecture becomes compromised. Activated fibroblasts sense the tissue damage through mechanosensitive channels, potentially upregulating collagen and proteoglycan production in an attempt at repair [397]. However, prolonged or severe overload can overwhelm these responses, resulting in macroscopic tears.

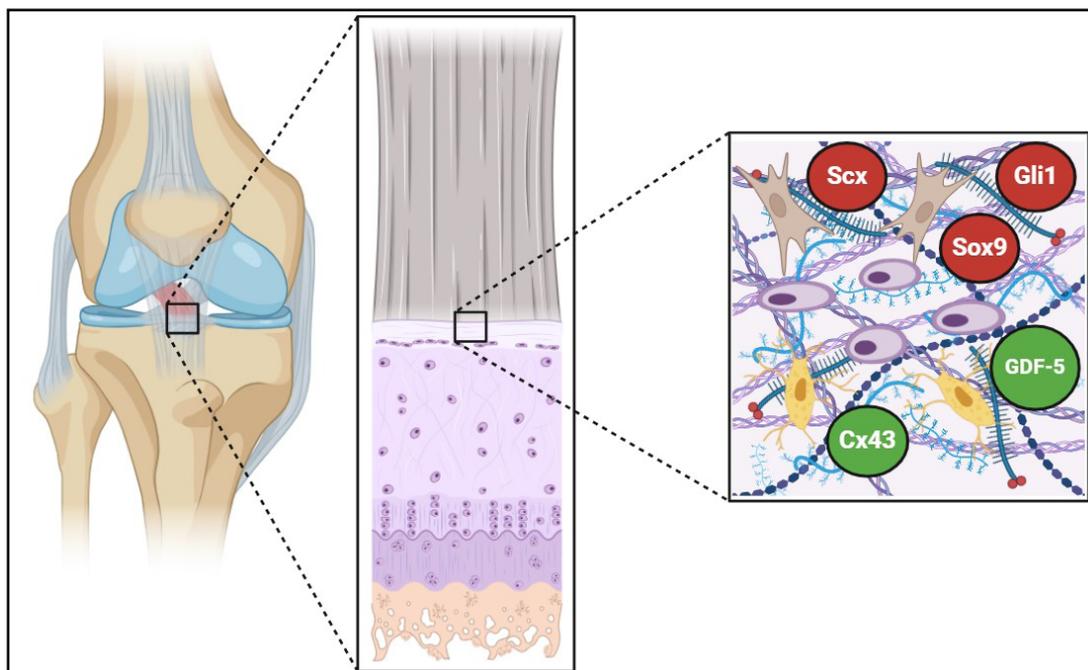
Ligaments do not remain static; they adapt over time and in response to metabolic factors [398]. Aging typically brings heightened collagen crosslink density, reduced proteoglycan content, and more frequent non-enzymatic glycation, causing ligaments to become stiffer and more prone to failure. Diabetes and other metabolic disorders can accelerate glycation, impairing ligament elasticity and contributing to joint dysfunction [399]. Moreover, age- or disease-related changes in ion channel expression can impair fibroblast mechanosensitivity, potentially diminishing the tissue's capacity to remodel and repair [400]. By recognizing how these molecular features intersect with mechanical load, researchers and clinicians gain critical insights for developing strategies—ranging from pharmacological modulation of ion channels to targeted physical therapies—that preserve ligament integrity and enhance long-term joint function.

## Ligament Enthesis: Molecular and Mechanical Transition

The enthesis is a highly specialized anatomical region where ligaments (and tendons) anchor into bone, enabling a seamless transition between soft and hard tissues (Fig. 6). This interface not only confers mechanical resilience by dispersing stress but also embodies a sophisticated molecular and cellular organization essential for tissue homeostasis and repair [401]. Emerging insights from molecular and cellular biology underscore the complexity of enthesis formation and maintenance, with transcription factors (e.g., Scleraxis/Scx, Sox9, Gli-1), growth factors (e.g., GDF-5), and gap junction proteins (Connexins) playing pivotal roles in controlling cell fate, ECM synthesis, and mechanotransduction [402, 403]. Scleraxis is a basic helix-loop-helix transcription factor that is widely regarded as a master regulator of tendon and ligament development. Its expression is predominantly associated with tendon/ligament progenitor cells, where it drives the synthesis of type I collagen and tenascin-C, among other tendon-specific ECM proteins [404]. In the context of the enthesis, Scx-expressing progenitor cells migrate toward the insertion site and initiate the formation of a dense collagen matrix. As mechanical loads intensify, Scx expression can be modulated by integrin-based signaling, ensuring that the enthesis region adapts collagen organization to withstand tensile forces [405].

Within the fibrocartilaginous region of the enthesis, the transcription factor Sox9 becomes increasingly important. Sox9 is traditionally recognized as a key regulator of chondrogenesis and is essential for the expression of type II collagen and aggrecan [406]. In the transitional zone of the enthesis—where fibrocartilage resides—Sox9 activity helps maintain a phenotype that is partly chondrogenic, providing resistance to compressive forces. This chondrocyte-like phenotype is crucial for reducing stress concentrations at the junction where the ligament transitions into mineralized tissue [407].

Recent studies point to a critical role of Hedgehog signaling in enthesis morphogenesis and maintenance, particularly via the transcription factor Gli-1. Gli-1 is a downstream effector of Sonic hedgehog (Shh) or Indian hedgehog (Ihh) signaling, orchestrating cell proliferation, differentiation, and survival in various skeletal tissues [408]. In the enthesis, Gli-1-positive cells can represent a progenitor population that contributes to ongoing remodeling. These progenitors respond to mechanical cues by modulating their differentiation into fibroblast-, chondrocyte-, or osteoblast-like cells, thus preserving the zonal composition of the ligament-to-bone interface [408, 409]. GDF-5, a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily, has garnered attention for its essential role in joint and tendon/ligament development. In the enthesis, GDF-5 is implicated in stimulating progenitor cell recruitment and chondrogenic differentiation, working synergistically with other factors such as bone



**Fig. 6.** Molecular Signaling in the Enthesis and Its Diverse Cell Populations. The enthesis is a biomechanically graded interface where tendons or ligaments merge seamlessly with bone. This transition occurs across distinct zones—fibrous, fibrocartilaginous, and mineralized—that are maintained by specialized cell populations (fibroblast-, chondrocyte-, and osteoblast-like cells, respectively). Numerous molecular signals guide the development, remodeling, and repair of these cell populations, ensuring that the enthesis can withstand multi-axial loading and mitigate stress concentrations. Transcription Factors and Cell Fate Scleraxis (Scx): A master regulator of tendon and ligament development, Scx is vital for synthesizing type I collagen and tendon-specific proteins. Its expression is regulated by mechanical cues via integrin-based signaling, promoting collagen organization in response to tensile forces. Sox9: Primarily associated with chondrogenesis, Sox9 drives the formation of type II collagen and aggrecan, giving fibrocartilage its compressive resistance. Within the enthesis, Sox9 fosters the chondrocyte-like phenotype essential for dampening stress at the transition to mineralized tissue. Gli-1: Functioning downstream of Hedgehog signals, Gli-1-positive progenitors can differentiate into fibroblasts, chondrocytes, or osteoblasts. This dynamic capacity preserves the zonal architecture, allowing ongoing adaptation to mechanical stimuli. Growth Factors and Their Roles GDF-5: A TGF- $\beta$  superfamily member, GDF-5 enhances progenitor cell recruitment and chondrogenic differentiation. In synergy with BMPs and FGFs, GDF-5 promotes the formation of fibrocartilage, proteoglycan deposition, and eventual mineralization—key for a stable enthesis. Other Signaling Molecules (e.g., TGF- $\beta$ , BMPs, FGFs): These gradients guide each enthesis zone by modulating anabolic and catabolic processes, harmonizing the production of specialized ECM components. Gap Junction Communication Connexins (Cx43): Gap junctions formed by connexins allow direct exchange of ions and small signaling molecules (e.g., cAMP, IP3) across cells. This intercellular communication is crucial for synchronizing responses to mechanical stress, coordinating ECM turnover, and preventing localized tissue failure. ECM Stratification and Mechanotransduction: ECM Composition: Type I collagen predominates in the tensile ligament region, while type II collagen, type X collagen, and proteoglycans (aggrecan) rise in the fibrocartilage zone, adapting to compressive loads. At the mineralized front, hydroxyapatite integrates with collagen, forming calcified fibrocartilage that transitions to bone. Mechanical Signaling: Integrin-based focal adhesions anchor cells to the ECM and convert mechanical stimuli into biochemical signals. These cues influence transcription factor activity (Scx, Sox9, Gli-1), balancing ECM synthesis and degradation and ensuring the tissue adapts to shifting load demands. Overall, the enthesis's mechanical resilience relies on finely tuned molecular crosstalk among various cell types. Transcription factors like Scx, Sox9, and Gli-1 orchestrate lineage-specific ECM production, while GDF-5 and other growth factors steer progenitor differentiation. Gap junctions enable rapid signal spread, creating a synchronized response to mechanical stress. Together, these molecular and cellular mechanisms preserve enthesal integrity, enabling fluid load transfer and robust tissue repair when injury occurs.)

morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) [410]. Experimental models suggest that GDF-5 can promote the formation of fibrocartilaginous zones, enhance proteoglycan deposition, and accelerate the maturation of the mineralized region, all of which are key to a stable enthesis structure [402].

Increasing evidence indicates that enthesis cells rely on gap junction-mediated communication to synchronize matrix synthesis and degradation across different zones. Connexins (particularly Connexin 43, Cx43) form gap junction channels that allow direct intercellular exchange of ions and small molecules, including second messengers like cyclic AMP (cAMP) and inositol triphosphate (IP3) [409]. This connectivity is thought to be crucial for propagating signals that inform cells of mechanical changes, orchestrating a collective response in matrix remodeling. Disruptions in gap junction signaling could lead to maladaptive remodeling and increased susceptibility to enthesopathy or enthesis-related injuries [403]. The ECM at the ligament enthesis is uniquely stratified to handle multiple loading modes. The ligament proper is dominated by type I collagen fibers, which confer tensile strength. Progressing toward the fibrocartilaginous zone, type II collagen, type X collagen, and proteoglycans like aggrecan become more abundant [401, 406]. These components increase the tissue's capacity to resist compressive forces. At the mineralized interface, hydroxyapatite crystals intercalate with collagen fibers, creating a calcified fibrocartilage region that gradually transitions into subchondral bone [405]. Each of these zones is maintained by a discrete population of cells—fibroblast-like in the ligament region, chondrocyte-like in the fibrocartilage, and osteoblast lineage cells in the bone—which are regulated by molecular gradients involving TGF- $\beta$ , FGFs, BMPs, and other signaling pathways [407]. The interplay between Scx, Sox9, Gli-1, and GDF-5 ensures that cells in each zone differentiate appropriately and produce ECM components best suited to the mechanical environment [408, 410].

The mechanical environment of the enthesis demands precise modulation of cell behavior. Mechanical loads generated by joint movement are transmitted to enthesis-resident cells, where they are converted into biochemical signals—a process known as mechanotransduction [404]. Integrin-based focal adhesions anchor the cytoskeleton to ECM fibers, enabling cells to sense tension or compression. These signals feed into transcriptional networks involving Scx, Sox9, and Gli-1, adjusting the balance between anabolic (ECM synthesis) and catabolic (ECM breakdown) activities [402].

Further coordination is facilitated by gap junctions composed of Connexins, which allow neighboring cells to share signaling molecules and ions [403]. This rapid intercellular communication ensures that cells within different zones of the enthesis respond cohesively to shifts in mechanical load, thereby preventing localized failure and tissue damage. Finite element analyses and *in vivo* imaging consistently illustrate that the enthesis is not an abrupt union of two tissues but rather a mechanically graded interface [407]. Such gradation distributes tensile, compressive, and shear forces more evenly across zones, thereby reducing stress concentrations at any single point [401]. The fibrocartilaginous region, in particular, acts as a stress buffer, aided by Sox9- and GDF-5-driven chondrogenic differentiation that boosts proteoglycan content and compressive strength [405, 410].

When mechanical loading patterns change—due to injury or altered joint mechanics—Scx-positive progenitors in the soft tissue region can be activated to reorganize collagen fiber orientation, while Gli-1 and Sox9 pathways adapt the fibrocartilaginous and bone regions to shifting loads [408, 409]. If these adaptive processes are dysregulated (as in enthesitis, osteoarthritis, or chronic overuse), pathological remodeling ensues and may compromise the integrity of the entire insertion site [402].

## Future Directions

Future research is poised to significantly expand our molecular and cellular understanding of ligament biology. Single-cell transcriptomics and advanced proteomic profiling will unravel the heterogeneity of ligament cell populations, identifying distinct subpopulations that

respond differently to mechanical stimuli [96–98, 343–345]. By examining how individual cells regulate integrin expression, growth factor receptors, and ECM-remodeling enzymes, researchers can pinpoint molecular targets that either enhance ligament resilience or drive degenerative changes. Similarly, high-throughput proteomic analyses can clarify how post-translational modifications (e.g., phosphorylation of FAK or other focal adhesion proteins) and epigenetic regulators (e.g., chromatin-remodeling complexes and miRNAs) shape cell fate decisions under various loading regimens [346–348].

Concurrent advances in imaging technologies will shed light on how these molecular modifications manifest in tissue architecture. Super-resolution microscopy, for instance, can provide nanoscale visualization of integrin clusters, focal adhesions, and cytoskeletal filaments, enabling researchers to link molecular signaling events directly to changes in cell shape and ECM organization [99–101, 349–351]. Second-harmonic generation imaging and other nonlinear optical methods can reveal collagen fiber arrangements in unprecedented detail, correlating molecular-level ECM synthesis and alignment with mechanical properties [352–354]. Such insights can guide the development of scaffolds and rehabilitation programs that precisely modulate cell–ECM interactions.

The integration of computational modeling and machine learning offers a powerful avenue for predicting ligament cell responses to various mechanical loads. By incorporating data from single-cell omics, proteomics, and high-resolution imaging, computational models can simulate how alterations in integrin-binding motifs, growth factor delivery, or microRNA expression affect tissue-level outcomes [102–104, 355–357]. Machine learning algorithms can identify complex patterns in these large datasets, allowing researchers and clinicians to forecast the trajectory of ligament healing, the effectiveness of specific therapeutic interventions, or the optimal mechanical environment for engineered constructs. Tailoring patient-specific rehabilitation protocols and graft-preconditioning strategies becomes feasible as these predictive tools advance, ultimately improving clinical outcomes. As we understand more about how molecular signaling pathways, cellular responses, and mechanical inputs interact, new therapeutic strategies will emerge. Better management of ligament injuries, more efficient ACL graft ligamentization, and refined regenerative medicine techniques will hinge on these molecular insights [358–360]. Clinicians may soon be able to prescribe precision rehabilitation programs that fine-tune mechanical loading parameters based on patient-specific molecular signatures. Similarly, tissue engineers can precondition grafts at the molecular level—overexpressing key transcription factors, adjusting microRNA profiles, or delivering growth factors—to ensure that implanted constructs rapidly adapt to the *in vivo* environment, producing a functional ligament capable of bearing physiological loads [361–363].

In essence, harnessing these molecular and cellular insights will redefine our approach to ligament repair and regeneration. By integrating cutting-edge omics data, imaging technologies, computational models, and molecular engineering tools, we can move toward a future where ligament injuries are managed with a level of precision and efficacy previously unattainable. This systems-level understanding will bridge the gap between basic mechanobiological research and clinical application, ultimately restoring musculoskeletal function and improving quality of life for patients with ligament injuries [364–366].

## Conclusion

This review underscores the complexity and precision with which ligament tissues maintain structural integrity, adapt to their mechanical environment, and respond to injury. At the center of ligament homeostasis lies a delicate interplay between mechanical stimuli, molecular signaling pathways, and cellular phenotypes. Ligament fibroblasts, or ligamentocytes, continuously interpret and translate mechanical load patterns into biochemical responses via integrin-mediated focal adhesions, mechanosensitive ion channels, and associated mechanotransduction networks. The downstream signaling cascades—MAPKs, PI3K/AKT, and others—fine-tune gene expression, guiding collagen fibrillogenesis,

ECM composition, and cytoskeletal organization. This robust molecular machinery ensures that ligaments are not passive structures but highly responsive tissues capable of dynamic remodeling.

Distinct from tendons, ligaments must accommodate multidirectional forces. Their unique ECM composition, richer in elastin and proteoglycans, provides compliance and the ability to absorb and dissipate complex stresses. Appropriate mechanical loading preserves collagen alignment and ECM integrity, stabilizing the ligament cell phenotype. Conversely, insufficient or excessive loading triggers inflammatory mediators, catabolic enzymes, and altered transcriptional and epigenetic landscapes, leading to degenerative changes, scar formation, and compromised mechanical properties. Stress shielding and repetitive microtrauma further highlight the importance of balanced mechanical cues. Without the correct level of tension, ligament fibroblasts cannot maintain load-bearing collagen networks, predisposing the tissue to fibrocartilaginous transformation, laxity, or sudden mechanical failure.

Armed with emerging insights into growth factor signaling, cytokine modulation, epigenetic control, and transcriptional regulation, researchers and clinicians can now envision more sophisticated interventions. By modulating integrin ligands, harnessing growth factor gradients, controlling microRNA expression, or applying gene editing tools, it becomes possible to “reprogram” tendon grafts to adopt ligamentous phenotypes, accelerate ACL graft ligamentization, or stabilize degenerative ligaments. Moreover, mechanical stimulation protocols informed by chronobiological principles—calibrating loading cycles rather than relying on static timelines—hold promise for more effective rehabilitation strategies. Such approaches recognize that tissue healing and adaptation depend not solely on the passage of time, but on the cumulative number of load-rest cycles, their intensity, and their coordination with the tissue’s molecular rhythms. These insights directly impact tissue engineering and regenerative medicine. Designing scaffolds equipped with integrin-binding motifs and mechanical cues, delivering controlled growth factors, and employing bioreactors that replicate physiological loading patterns can produce constructs that emulate native ligament properties at the molecular, cellular, and structural levels. By preconditioning cells with genetic or epigenetic interventions, scaffolds can be “tuned” to ensure optimal ECM organization, mechanical resilience, and integration with host tissues post-implantation.

Looking toward the future, advanced single-cell transcriptomics, proteomics, and imaging technologies will deepen our comprehension of ligament cell heterogeneity and molecular dynamics. Combined with computational modeling and machine learning, these data-rich approaches will enable predictive modeling of healing trajectories and patient-specific therapies. Clinicians may soon customize rehabilitation protocols based on molecular “signatures” of a patient’s ligament cells, optimizing load regimens to prevent degenerative cascades and enhance repair. However, it is important to note that there is no single universally optimal mechanical loading regimen; instead, a deep understanding of cellular biology can enhance clinicians’ confidence in selecting targeted rehabilitation approaches and preventing future injuries.

Ultimately, the convergence of molecular mechanobiology, engineering principles, and clinical insight is poised to transform ligament care. However, it is important to note that these concepts remain largely theoretical, as the majority of supporting evidence currently comes from animal models or laboratory-based studies, which may not fully reflect the complexities of human physiology and clinical practice. By bridging fundamental research and clinical application, we can foster a new era of precision medicine in orthopedics—one in which ligament injuries are managed through biologically informed, biomechanically sound strategies that restore function, reduce reinjury risk, and significantly improve patient outcomes.

## Abbreviations

ECM (Extracellular Matrix); FAK (Focal Adhesion Kinase); MAPKs (Mitogen-Activated Protein Kinases); PI3K/AKT (Signal Pathways); GAGs (Glycosaminoglycans); SLRPs (Small Leucine-Rich Proteoglycans); TGF- $\beta$  (Transforming Growth Factor Beta isoforms); FGF

(Fibroblast Growth Factor); CTGF (Connective Tissue Growth Factor); MMPs (Matrix Metalloproteinases); TIMPs (Tissue Inhibitors of Metalloproteinases); Scx (Scleraxis Transcription Factor); Mxk (Mohawk Transcription Factor); EGR1 (Early Growth Response 1); GDFs (Growth Differentiation Factors); TRP (Transient Receptor Potential); VEGF (Vascular Endothelial Growth Factor); TFs (Transcription Factors); IGF-1 (Insulin-like Growth Factor 1); NO (Nitric Oxide); ROS (Reactive Oxygen Species); AGEs (Advanced Glycation End Products)

## Disclosure Statement

The authors have no conflicts of interest to declare.

### *Declaration of AI usage*

During the preparation of this work, the authors used Grammarly to improve readability and language quality.

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