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Mechanobiology of the Meniscus

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Abstract

The meniscus plays a critical biomechanical role in the knee, providing load support, joint stability, and congruity. Importantly, growing evidence indicates that the mechanobiologic response of meniscal cells plays a critical role in the physiologic, pathologic, and repair responses of the meniscus. Here we review experimental and theoretical studies that have begun to directly measure the biomechanical effects of joint loading on the meniscus under physiologic and pathologic conditions, showing that the menisci are exposed to high contact stresses, resulting in a complex and nonuniform stress-strain environment within the tissue. By combining microscale measurements of the mechanical properties of meniscal cells and their pericellular and extracellular matrix regions, theoretical and experimental models indicate that the cells in the meniscus are exposed to a complex and inhomogeneous environment of stress, strain, fluid pressure, fluid flow, and a variety of physicochemical factors. Studies across a range of culture systems from isolated cells to tissues have revealed that the biological response of meniscal cells is directly influenced by physical factors, such as tension, compression, and hydrostatic pressure. In addition, these studies have provided new insights into the mechanotransduction mechanisms by which physical signals are converted into metabolic or pro/anti-inflammatory responses. Taken together, these *in vivo* and *in vitro* studies show that mechanical factors play an important role in the health, degeneration, and regeneration of the meniscus. A more thorough understanding of the mechanobiologic responses of the meniscus will hopefully lead to therapeutic approaches to prevent degeneration and enhance repair of the meniscus.

Keywords

mechanical signal transduction; articular cartilage; collagen; fibrochondrocyte; proteoglycan

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Conflict of interest statement

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1. Introduction

The menisci are fibrocartilaginous tissues that play a critical role for the transmission and distribution of loads in the knee (Walker and Erkman, 1975). The unique properties of the menisci are determined by the complex geometry, ultrastructure, and composition of the tissue, which consists of a hydrated extracellular matrix composed largely of collagen, which varies in type from predominantly type II collagen in the more cartilaginous inner zone, to primarily type I collagen in the vascularized, fibrous outer zone (Fithian et al., 1990; Makris et al., 2011). The remainder of the solid matrix includes smaller amounts of other collagens, proteoglycans, proteins, and glycoproteins (McDevitt and Webber, 1990). Injury or degeneration of the meniscus is associated with pain and joint dysfunction, and loss of meniscal function or surgical meniscectomy leads to relatively rapid and progressive osteoarthritis (Fairbank, 1948). Partial meniscectomy is often utilized to treat meniscal tears; however, despite improvements in pain and function, this surgery does not protect against the development of osteoarthritis (Andersson-Molina et al., 2002; Hall et al., 2014; Hoser et al., 2001). In addition, the menisci show little capacity for repair, except for certain types of injuries occurring in the peripheral vascularized region (Arnoczky and Warren, 1983; Scott et al., 1986).

The composition and structure of the meniscus is maintained through a balance of the anabolic and catabolic activities of the residing cells, often termed “fibrochondrocytes.” The biological activity of meniscal cells is controlled not only by genetic and biochemical factors, such as growth factors and cytokines (Collier and Ghosh, 1995; McNulty et al., 2013; Pangborn and Athanasiou, 2005; Riera et al., 2011), but also by physical factors associated with joint loading. It is now clear that mechanical factors play a critical role in the development, maintenance, degeneration, and repair of the meniscus. In this regard, a thorough understanding of the mechanobiology of the meniscus under physiologic and pathologic loading conditions may provide important insights into the prevention and treatment of meniscal injuries and degeneration.

A range of different approaches have been used to study the mechanobiologic responses of the meniscus, ranging from *in vivo* studies to the cell and molecular level, with each type of study providing certain advantages and disadvantages. *In vivo* animal studies generally represent the most physiologically relevant model systems and can provide a means for studying long-term (i.e., weeks to years) effects associated with development, remodeling, or repair. *In vivo* studies are generally limited by the complexities involved in determining the precise mechanical environment of the menisci, and may be further complicated by the effect of systemic factors. At the tissue level, *in vitro* studies can provide important information on the mechanobiologic regulation of meniscal cells, where both the applied load and biochemical environment can be better controlled. These studies have generally focused on meniscal explants or isolated cells grown in three-dimensional (3D) matrices. In explant culture, the native cell-matrix interactions are maintained; however, in cartilaginous tissues, the presence of the extracellular matrix generates other physical signals associated with applied loading that can vary significantly with time and at different sites in the tissue. Thus, many of the biophysical phenomena that may be directly responsible for regulating meniscal cell responses cannot be uncoupled in a tissue explant model. Thus, studies on

isolated cells can provide model systems for studying specific signal transduction pathways or for isolating the effects of a single biophysical stimulus, such as stretch or hydrostatic pressure.

In this paper, we present a review of the mechanobiology of the meniscus – that is, the influence of mechanical factors on the biological response of meniscal cells. These studies have been performed in a range of model systems across different geometric scales and the interpretation of these studies has been greatly enhanced by the use of theoretical and experimental models designed to predict and quantify the mechanical environment of cells in the meniscus under different loading conditions. Furthermore, several *in vivo* and *in vitro* studies have begun to examine the influence of mechanical factors on meniscal regeneration and repair. Together, these studies further our understanding of the role of mechanobiology in the development, growth, maintenance, degeneration, and repair of the meniscus.

2. *In vivo* studies of meniscal mechanobiology

In the 19th century, it was generally believed that the menisci were inert vestigial tissues that were remnants of intra-articular muscles (Bland-Sutton, 1897). However, classical studies by Fairbank (Fairbank, 1948) and numerous more recent studies (Roos et al., 2001) have shown distinct and repeatable deleterious changes in the knee joint following loss of the meniscus. It is now apparent that not only is the meniscus a critical functional element of the knee joint, it contains multiple subpopulations of active cells that are responsible for tissue development, maintenance, and repair (Hellio Le Graverand et al., 2001; Verdonk et al., 2005). The specific morphology and arrangement of the meniscal cells may play a role in their ability to respond to different types of mechanical signals experienced throughout the tissue. Importantly, growing evidence has shown that these cells are highly responsive to the local biophysical environment under both physiologic and pathologic conditions, and that changes in the loading history of the joint can alter meniscal composition, structure, and inflammatory response.

Much of the initial data on the mechanosensitivity of the meniscus came from *in vivo* studies of joint immobilization. Immobilization was found to lead to a “disuse atrophy” that was characterized by the loss of proteoglycans in the meniscus (Klein et al., 1982; Videman et al., 1979). These changes are apparent histologically, as well as at the gene expression level (Djurasovic et al., 1998) and are also reflected in altered mechanical properties and function of the meniscus (Anderson et al., 1993; Ochi et al., 1997). Further evidence for the mechanosensitive behavior of the meniscal cells was shown by the fact that loss of the extracellular matrix components can be prevented through muscular stimulation in cast-immobilized joints (Burr et al., 1984) or through active motion without direct weight-bearing (Klein et al., 1989). Interestingly, limb immobilization in the chick embryo prevented the formation of the meniscus, indicating the critical role of mechanobiology in meniscal development (Mikic et al., 2000). Furthermore, exercise has been shown to alter the composition of the meniscus. For example, treadmill running caused a significant decrease in dermatan sulfate proteoglycans, as well as the number of pyridinoline crosslinks per mole of collagen in the menisci of chickens (Pedrini-Mille et al., 1988). These effects were age-dependent, and only occurred during the period of active growth. In rats, strenuous

treadmill running was found to cause changes in the hydroxyproline and calcium contents of the lateral meniscus, but only in the posterior region (Vailas et al., 1986).

Consequently, it is not surprising that mechanical factors can influence meniscal repair *in vivo*. For example, in a dog model, lesions in the vascular region of the medial meniscus exhibited decreased collagen content in animals that were cast immobilized, as compared to animals that were mobilized immediately after surgery (Dowdy et al., 1995). While the mechanisms involved in this response are not well understood, other studies in a rabbit model showed that injury to the medial meniscus was characterized by a five-fold increase in blood flow to the menisci 4 weeks post-operatively. However, immobilization of the knee joint prevented this increase (Bray et al., 2001). Furthermore, *in vivo* studies have shown that mechanical loading can drive either anti-inflammatory or pro-inflammatory responses of meniscal cells. In a rabbit model of antigen-induced arthritis, continuous passive motion was found to prevent proteoglycan degradation and loss from the meniscus, as compared to immobilized knees (Ferretti et al., 2005). Interestingly, continuous passive motion induced an anti-inflammatory response, characterized by increased levels of the cytokine interleukin (IL)-10 in meniscal fibrochondrocytes. Conversely, injurious joint loading in a tibiofemoral impaction model in the rabbit has been shown to result in a significant decrease in cell viability in the lateral menisci following injury, and a trend towards increased pro-inflammatory mediators, such as nitric oxide (NO) (Killian et al., 2014). Taken together, these *in vivo* studies emphasize the importance of physiologic mechanical loading in the health and function of the meniscus and consequently the entire knee joint, and indicate that abnormal loading can result in catabolic and pro-inflammatory responses by meniscal cells.

3. Mechanical environment at the tissue and cellular level

From these *in vivo* studies, it is apparent that the mechanical loading history of the knee has a strong influence on the metabolic activity of cells within the meniscus. However, the precise mechanisms involved in regulating the synthesis and breakdown of meniscal matrix components are not fully understood. A critical factor in deciphering the mechanisms of meniscal cell response to joint loading is an understanding of the stress-strain environment of the meniscus and surrounding tissues under physiologic or pathologic loading conditions. However, due to the inhomogeneous composition and the anisotropic structure and mechanical properties of the meniscus, the local stress-strain and fluid flow environments may vary greatly with time and location. Furthermore, the charged and hydrated properties of the tissue result in complex physicochemical, electrical, and fluid flow environments, in addition to tensile, compressive, and shear stresses and strains. Thus, it has been difficult to isolate the effects of specific biophysical factors on meniscal cell activity due to the intrinsic coupling of these various phenomena.

Thus, to better understand the biophysical environment of the meniscus, a number of studies have used minimally-invasive imaging modalities, such as magnetic resonance imaging (MRI) or roentgen stereophotogrammetric analysis (RSA) to measure meniscal deformation *in situ.* In human knees, both the lateral and medial menisci were found to undergo large movements, as well as morphologic changes, during knee joint flexion (Kawahara et al., 1999; Tienen et al., 2005; Vedi et al., 1999). Furthermore, following running, menisci

showed a significant loss of volume that was dependent on the duration of exercise (Kessler et al., 2006), and both T1rho and T2 MR signal intensities were found to increase after a marathon, indicative of persistent matrix changes (Stehling et al., 2011). More recently, volume registration of MRI images were used to examine 3D displacement and local strain in the meniscus of pig knees. This study found that the meniscus and its attachments showed low average radial or circumferential stretch (less than 1%), but axial strains of nearly 12% during physiologically relevant loading of the knee (Figure 1) (Freutel et al., 2014).

To determine the internal stress-strain and fluid pressure environment, computational methods such as finite element modeling are necessary in combination with appropriate constitutive and geometric representations of the meniscus. Such models have provided important insights on the mechanical environment of the meniscus under different loading conditions and have shown that the predicted stress and strain fields were highly dependent on the assumed mechanical properties of the tissue, and thus could exhibit significant inhomogeneity and anisotropy (Meakin et al., 2003; Spilker et al., 1992; Yao et al., 2006). The incorporation of fluid pressurization effects through a biphasic or poroelastic model were found to be necessary to account for the physiologic load-bearing capacity of the meniscus (LeRoux and Setton, 2002; Spilker et al., 1992). Furthermore, damage or partial loss of the meniscus or knee ligaments can greatly influence the displacement and deformation of the meniscus under load (Yao et al., 2006). Importantly, the predictions of such models have been confirmed or validated directly using MRI and pressure sensor measurements (Bedi et al., 2010; Halonen et al., 2014).

To better understand the mechanobiologic response of meniscal cells, several studies have developed novel computational or experimental systems to predict or measure the relationship between the macroscale mechanical environment of the meniscus and the local microscale environment around individual cells. At the tissue level, the meniscus has been shown to possess distinct depth-dependent strain profiles during compression (Lai and Levenston, 2010) and the transfer of strains to the local level have been shown to exhibit considerable spatial heterogeneity, with evidence of both strain amplification and attenuation, depending on the site within the meniscus (Han et al., 2013; Upton et al., 2008). These characteristics are consistent with a complex mechanical environment that may arise from fiber sliding, fiber twisting, and fiber-matrix interactions that are present in the meniscus.

While such experimental studies and standard finite element models of meniscus mechanics have provided important information on the spatial and temporal stress-strain environment at the microscale tissue level, the mechanical environment at the cellular level will depend on other factors, including the structure and mechanical properties of the pericellular matrix, as well as those of individual cells (Guilak and Mow, 2000). In the past decade, several studies have begun to report such properties and their application in finite element models of cell-matrix interactions in the meniscus. The first measure of meniscal cell mechanical properties was performed using micropipette aspiration of isolated cells, which showed a mean instantaneous Young's modulus of ~0.5 kPa and an equilibrium modulus of ~0.2 kPa (Upton et al., 2006). In other studies, bulk cell compression was used to measure the properties of meniscal cells, showing that they can differ significantly between inner and outer zone cells

(Sanchez-Adams and Athanasiou, 2012). By incorporating such cell mechanical properties, along with meniscal cell geometries, into a nonlinear anisotropic biphasic model, finite element studies have begun to examine the relationship between extracellular matrix strain and cell strain, showing significant regional differences in the cellular mechanical environment in response to biaxial tissue strains (Figure 2) (Upton et al., 2006). In other studies, a 2D, non-linear, fiber reinforced, multi-scale finite element model was utilized to quantify changes in the stress, strain, fluid velocity and fluid flow induced shear stress in the vicinity of meniscal cells (Gupta and Haut Donahue, 2006). This study showed that not only did cell shape and location significantly influence the mechanical environment, but that the pericellular matrix could play a significant role in shielding the cell from large principal strains and stresses. While the presence of a pericellular region had been observed previously in the meniscus (McDevitt et al., 2001), only recently have the microscale properties of this region been investigated. By combining atomic force microscopy (AFM) with simultaneous fluorescence imaging of perlecan to demarcate the pericellular matrix, the mechanical properties of matched pericellular and extracellular matrix sites were spatially mapped within the outer, middle, and inner porcine medial meniscus (Sanchez-Adams et al., 2013). This study showed that the elastic modulus of the meniscus pericellular matrix was significantly higher in the outer region (~150 kPa) than the inner region (~28 kPa) (Figure 3), whereas extracellular matrix moduli were consistently higher than region-matched pericellular matrices in both the outer (~321 kPa) and inner (~66 kPa) regions. These findings indicate that the structure and composition of the tissue can regulate the local stress-strain environment of meniscal cells among the different regions of the tissue (Sanchez-Adams et al., 2013).

4. Explant and 3D studies of meniscal mechanobiology

The effects of controlled mechanical loading regimes have been assessed on tissue explants, which maintain the meniscal cells in the context of the native extracellular matrix. Mechanical loading effects on meniscal explants depend on the strain and magnitude of the applied load, the frequency, and the type of load applied. In general, dynamic compression tends to be anabolic at 10% strain but then the balance shifts towards catabolism at 20% strain (Gupta et al., 2008; Zielinska et al., 2009). In particular, the physiologic strain of 10% for 2 hours at 1Hz suppressed the release of the pro-inflammatory mediator NO (Gupta et al., 2008) and enhanced aggrecan gene expression (Aufderheide and Athanasiou, 2006) but also increased the catabolic genes cyclooxygenase-2 (COX2) and a disintegrin and metalloproteinase with thrombospondin (ADAMTS)-5 (Zielinska et al., 2009). However, higher strain magnitudes of 20% induced a pathologic response characterized by the upregulation of matrix metalloproteinase (MMP)-1, MMP-3, MMP-13, ADAMTS-4, inducible nitric oxide synthase (NOS2), and interleukin-1α (IL-1α) mRNA, and consequently resulted in increased NO release and the breakdown of proteoglycans (Gupta et al., 2008; McHenry et al., 2006; Zielinska et al., 2009). On the other hand, the expression of type I collagen, tissue inhibitor of metalloproteinases (TIMP)-1, and transforming growth factor (TGF)-β were not regulated by mechanical loading of meniscal explants between 0 and 20% dynamic compression (Zielinska et al., 2009).

In addition to determining the effects of physiologic strain on meniscal tissue explants, overloading models elucidate the response of the tissue to injurious loads. The application of 40% strain to immature bovine meniscal explants at increasing strain rates (0.5%/s, 5%/s, and 50%/s) induced biological but not physical or compositional changes in the meniscus tissue (Nishimuta and Levenston, 2012). Cell metabolism was lowest at day 1 following overloading for the two highest strain rates tested and cell lysis was correlated with loading rate and peak injury force. However, there was no macroscopic damage to the tissue and the sulfated glycosaminoglycan (sGAG) content of the tissue, sGAG release, and mechanical properties were unaffected. Overloading of inner zone meniscal explants with a single insult of 50% strain at 100% strain/s caused large regions of cell death at the surface of the tissue, but surprisingly there was no gross damage to the tissue and downregulation of many catabolic and pro-inflammatory genes (Kisiday et al., 2010).

The loading configuration and history can also differentially regulate meniscal cell responses. For example, static compressive stress of 0.1 MPa for 24 hours downregulated types I and II collagen and decorin and increased MMP-1 gene expression, while dynamic stress suppressed the expression of decorin and type II collagen (Upton et al., 2003). On the other hand, oscillatory tension at 1 Hz and 10% displacement of immature bovine meniscal cells in 3D fibrin constructs decreased DNA content, sGAG content, and ^{35}S-sulfate incorporation (Vanderploeg et al., 2004). Over time in culture, the meniscal cells developed a stellate morphology and organized cytoskeletal filaments composed of F-actin, vimentin, and vinculin filaments; however, loading had no effects on the cytoskeletal morphology of the cells. Hydrostatic pressure can also regulate the metabolic activities of meniscal cells. The static application of 4 MPa of hydrostatic pressure for 4 hours to human meniscal cells in alginate beads resulted in the suppression of MMP-1 and MMP-13 mRNA levels (Suzuki et al., 2006). Conversely, cyclic hydrostatic pressure at 1 Hz resulted in the upregulation of mRNA transcripts for type I collagen, TIMP-1, and TIMP-2. The culture of rabbit meniscal explants increased catabolic gene expression, including mRNAs for MMP-1, MMP-3, TIMPs, NOS2, COX2, IL-1β, and IL-6 (Natsu-Ume et al., 2005). However, the application of 1 MPa cyclic hydrostatic pressure at 0.5 Hz for 4 hours prevented these culture-induced increases in catabolic gene expression. These studies reveal that the response of the meniscus to mechanical loading is at least partially regulated at the transcriptional level (Upton et al., 2003) and that mechanical loading can maintain meniscal tissue homeostasis during culture (Natsu-Ume et al., 2005).

Few studies have explored the interaction of growth factors and mechanical loading on meniscal explants. TGF-β1, insulin-like growth factor (IGF)-1, and platelet derived growth factor (PDGF)-AB increased both protein and proteoglycan production by immature bovine meniscal explants (Imler et al., 2004). In the presence of these growth factors, 25 – 50 % static compression for 4 days caused a suppression of matrix production equivalent to uncompressed controls, suggesting that the growth factors and mechanical stimulation may modulate meniscal metabolism through separate pathways. While TGF-β is not regulated at the transcriptional level by 10 – 20% dynamic compression (Zielinska et al., 2009), the combined treatment of meniscal explants with growth factors and dynamic compression has not yet been reported.

Joint injuries and arthritis affect both the biomechanical and the biochemical environment of the menisci. Following joint injury and in arthritic joints, the pro-inflammatory cytokine IL-1 is upregulated, with median synovial fluid concentrations rising to 109 pg/mL in mild osteoarthritic joints and 288 pg/mL in moderate osteoarthritic joints (McNulty et al., 2013). A number of studies have elucidated an interaction between pro-inflammatory pathways and mechanical signaling in meniscal explants. Dynamic compression of porcine meniscal explants at 0.1 MPa and 0.5 Hz for 24 hours increased NOS2 protein levels, NO production (Fink et al., 2001), protein and proteoglycan synthesis (Shin et al., 2003), and proteoglycan release. In addition, dynamic compression ranging from 0.0125 to 0.5 MPa elevated prostaglandin E_2 (PGE2) and NO production in a stress magnitude dependent manner (Hennerbichler et al., 2007). NOS2 and NOS3 protein production was also increased by mechanical stimulation, and NO inhibitors suppressed the mechanically induced NO production (Hennerbichler et al., 2007). IL-1 blocked the compression induced increases in protein and proteoglycan synthesis; however, these increases were restored by the NOS2 inhibitor 1400W (Shin et al., 2003). IL-1 also increased proteoglycan release and synergized with dynamic compression to further enhance release in a manner that was dependent on NO, specifically NOS2. Therefore, IL-1 modulates the effects of mechanical stress on meniscal tissue turnover via an NO dependent pathway (Shin et al., 2003). Pro-inflammatory and catabolic genes, such as IL-1α, MMP-1, MMP-3, and MMP-13, and sGAG release were increased by 20% dynamic compression at 1 Hz for 2 hours in young porcine meniscal explants (Killian et al., 2011). However, in the presence of IL-1 receptor antagonist (IL-1ra), the pro-inflammatory and catabolic genes were suppressed, as compared to compression alone. Therefore, with altered joint loading due to injury or arthritis, the meniscus likely serves as a source of pro-inflammatory mediators.

5. 2D cell stretching studies

The interplay between mechanical stimulation and inflammation is clearly established in explant model systems, however the specific downstream signaling pathways involved in meniscal mechanobiology can more easily be evaluated in 2D isolated meniscal cell cultures. For example, cyclic tensile strain has been shown to suppress the upregulation of NOS2, MMP-13, and TNF-α by IL-1β in a magnitude-dependent fashion, with greater than 90% suppression at 15% strain (Ferretti et al., 2006). Interestingly, these effects were frequency-dependent, such that higher frequencies more effectively suppressed IL-1 mediated catabolism. Additionally, the anti-catabolic effects of mechanical loading were sustained up to 24 hours after biomechanical stimulation, even in the continued presence of IL-1, suggesting the utility of biomechanical signals to block the catabolic effects of chronic inflammation following a joint injury or during arthritis.

While the mechanism of this response is not fully understood, cyclic tensile strain appears to antagonize IL-1 signaling through pathways upstream of transcription (Agarwal et al., 2001). Meniscal cells constitutively expressed low levels of receptor activator of nuclear factor (NF-κB) ligand (RANKL) and its cell surface receptor, RANK, but marked levels of the soluble neutralizing receptor osteoprotegerin (OPG) (Figure 4A) (Deschner et al., 2006). IL-1β treatment caused the upregulation of RANK and RANKL but did not alter OPG expression. Cyclic tensile strain suppressed the IL-1 mediated upregulation of RANK and

RANKL at both the mRNA (Figure 4B) and protein levels (Figure 4C, D) in a sustained, magnitude and frequency-dependent fashion. Therefore, mechanical stimulation utilizes the NF-κB signaling pathway to modulate the response to inflammation and regulate the transcription of pro-inflammatory mediators.

In other studies, TNF-α increased NO production and total protein synthesis, while suppressing proteoglycan synthesis in porcine meniscal cells from the inner zone (Fermor et al., 2004). The combination of 5% tensile strain at 0.5 Hz for 24 hours and TNF-α suppressed the strain-dependent increase in proteoglycan synthesis, in a NOS2 independent fashion. This study provides evidence that inflammatory cytokines can modulate the response of meniscal cells to mechanical stimulation.

The distinct phenotypes of the cells in the inner and outer zones of the meniscal tissue likely play a role in their response to mechanical stimulation. In unloaded cells, mRNA levels for decorin and biglycan and total protein and NO production were similar between isolated inner and outer zone meniscal cells (Upton et al., 2006). In contrast, inner zone cells had higher mRNA levels of type II collagen, aggrecan, and NOS2 and higher rates of proteoglycan synthesis and outer zone cells had higher mRNA levels for type I collagen. The application of 5% biaxial tensile strain at 0.5 Hz increased total protein synthesis and NO production for both inner and outer zone cells. However, there were no strain-induced changes in proteoglycan synthesis or gene expression (Upton et al., 2006). In contrast, type II collagen and SOX-9 were detectable in human osteoarthritic inner zone meniscal cells, but not in outer zone cells, and were upregulated at both the mRNA and protein levels by 5% uniaxial cyclic tensile strain at 0.5 Hz (Kanazawa et al., 2012). In the inner zone cells, stretch stimulated nuclear translocation and phosphorylation of SOX-9 and increased the association of SOX-9 with its binding site on the Col2a1 enhancer in chromatin, suggesting a role for mechanical stimulation in the epigenetic regulation of type II collagen expression (Kanazawa et al., 2012). Uniaxial tensile strain also upregulated the expression of the multifunctional growth factor CYR61/CTGF/NOV (CCN)-2 and type I collagen in inner zone meniscal cells but not outer zone cells (Furumatsu et al., 2012). Therefore, in addition to differences in the gene expression profiles of the inner and outer zones cells, meniscal cells from the two zones respond differently to mechanical stimulation.

6. Mechanisms of mechanotransduction

While the mechanobiologic response of meniscal cells has begun to be studied by a number of researchers, the mechanisms involved in mechanotransduction are still not well understood and remain to be elucidated. Many of the candidate mechanotransduction pathways examined in meniscal cells are based on those found in other cartilaginous cells, such as articular chondrocytes. For example, oscillatory fluid flow induced shear stresses ranging from 0 – 6.5 Pa caused an increase in intracellular Ca^{2+} and sGAG production in isolated rabbit meniscal cells (Eifler et al., 2006). The percentage of meniscal cells responding with a Ca^{2+} signal was positively correlated with the applied stress. Treatment of the mechanically stimulated cells with thapsigargin blocked both Ca^{2+} signaling and sGAG production (Eifler et al., 2006), suggesting that mechanically induced Ca^{2+} signaling may modulate meniscal extracellular matrix biosynthesis. However, the transporters and/or

receptors that are responsible for sensing the mechanical load and transporting the Ca^{2+} into the meniscal cells have yet to be identified. Recent advances have allowed the real time assessment of Ca^{2+} signaling during micromechanical loading on a confocal microscope (Han et al., 2014). Tensile deformation (0 - 9%) was applied to juvenile bovine outer zone meniscal cells either *in situ*, in aligned poly(ε-caprolactone) (PCL) scaffolds, or on silicone membranes (Han et al., 2014). Mechanical stimulation increased the amplitude of the peak Ca^{2+} signal in the meniscal cells in the context of the native tissue but not in meniscal cells on the scaffold or silicone membrane. In the native tissue, the percent of responding cells increased linearly from 0 - 9% strain, whereas in the scaffolds and on the silicone membrane the percent of responding cells increased linearly at a lower range of applied strains (< 3% strain) (Han et al., 2014). In summary, this study showed that the meniscal cells in native tissue and tissue engineered constructs respond differently to mechanical stimulation and thus the Ca^{2+} signaling profile in native meniscal tissue provides benchmarks for mechanoregulation during the generation of tissue engineered constructs.

7. Role of mechanical loading in tissue engineering and repair of the meniscus

Our understanding of the influence of mechanical factors on meniscal metabolism can be exploited to promote meniscal regeneration and repair. Therefore, tissue engineering strategies, which involve the combination of cells, scaffolds, growth factors, and bioreactors, are being explored to regenerate new meniscal tissue for the replacement of injured and/or degenerated meniscal tissue (AufderHeide and Athanasiou, 2004; Guilak et al., 2014). Current work focuses on determining the optimal cell types, scaffolds, growth factors, type of mechanical stimulation, and loading regimes to generate tissue engineered meniscal constructs that reach the biomechanical benchmarks of native meniscal tissue (Setton et al., 1999). Perfusion culture has been utilized to help promote diffusion into tissue engineered constructs. An optimal flow rate of 40 mL/min was determined for cell viability and extracellular matrix production, using polyethylene terephthalate scaffolds seeded with ovine fibrochondrocytes, as lower flow rates led to poor cell growth and higher rates of flow were destructive (Neves et al., 2002). However, oxygen deprivation in the center of the constructs was still problematic. Therefore, subsequent studies have utilized both perfusion and mechanical compression to generate tissue engineered meniscal constructs. Collagen meniscus implants seeded with human bone marrow stem cells (MSCs) showed increased procollagen types I and III peptide and construct mechanical properties with 10 mL/min perfusion and 10% dynamic strain at 0.5 Hz (Petri et al., 2012). Perfusion combined with shorter bouts of 10% dynamic strain at 0.5 Hz also increased the equilibrium modulus 1.85-fold and increased type I procollagen and extracellular matrix deposition in porous polyurethane scaffolds seeded with human MSCs (Liu et al., 2012). In addition, cell viability, density, and proliferation were enhanced with perfusion and strain, as was the tensile modulus, which was increased two-fold to approximately 1 MPa at 1 week. While dynamic compression and perfusion increased the mechanical properties of tissue engineered constructs, these scaffolds do not yet approach the tensile modulus of ~100 MPa and the equilibrium compressive modulus of ~200 kPa for native meniscal tissue (Setton et al., 1999).

Dynamic compressive loading of anatomically shaped scaffolds has also shown promise in the generation of tissue engineered meniscal constructs. Immature bovine meniscal cells were seeded into 2% alginate and crosslinked in meniscus shaped molds and then loaded by unconfined compression at 15% strain and 1 Hz 3 times/week for up to 6 weeks using anatomically shaped loading platens (Ballyns and Bonassar, 2011). After only 2 weeks of dynamic compression, the extracellular matrix content was enhanced and the compressive modulus was approximately 70% of the native meniscal tissue. Interestingly, 2 weeks of loading followed by 4 weeks of static culture resulted in a more mature matrix, including increased collagen bundle formation, sGAG content, collagen content, and 4.3-fold increased compressive equilibrium modulus (Puetzer et al., 2012). The simultaneous application of dynamic compression at 10% strain and 1 Hz for 1 hour/day and circumferential tension loading has also been assessed, using anatomically wedge shaped self-assembly meniscal constructs composed of young bovine articular chondrocytes and meniscal cells (Huey and Athanasiou, 2011). With early mechanical loading from days 10-14, the compressive and tensile moduli were enhanced slightly and further increased 3 to 6-fold by treatment with chondroitinase ABC and TGF-β1 in combination with mechanical stimulation. However, in each of these studies the tissue engineered construct properties are still less than the native mechanical properties.

In addition to the optimization of different loading regimes to promote meniscal tissue regeneration, various techniques have also been utilized to direct the deposition of extracellular matrix components within the scaffolds. Bacterial cellulose scaffolds that contain 500 μm diameter microchannels promoted the alignment of 3T6 mouse fibroblasts and also collagen fiber alignment within the scaffold (Martinez et al., 2012). The application of 5% dynamic compression at 0.1 Hz enhanced collagen production on the scaffolds as well. Aligned and randomly organized electrospun PCL scaffolds have also been utilized to control cell morphology and matrix deposition in meniscus tissue engineered constructs (Nathan et al., 2011). The cell nuclei aligned with the predominant scaffold fiber orientation. In addition, the application of up to 10% tensile strain of aligned scaffolds further enhanced the orientation and elongation of cells (increased the nuclear aspect ratio) in the direction of fiber alignment or the nuclei reoriented in the direction of the applied load. Furthermore, actin was necessary for the loading-induced changes in the nuclear aspect ratio, while microtubules and intermediate filaments were not essential for this response. Interestingly human MSCs were more pliable and responsive to these biophysical cues than differentiated osteoarthritic meniscal cells, suggesting that biophysical methods can be utilized to promote tissue maturation with stem cells. However, the infiltration of cells throughout electrospun scaffolds is also challenging. Recently, the use of an orbital shaker at 1.2 Hz improved cellular infiltration of young bovine MSCs, increased collagen content, and the uniform distribution of collagen throughout the constructs but suppressed sGAG content, relative to free swelling conditions (Nerurkar et al., 2011). On the other hand 6 weeks of dynamic culture followed by 6 weeks of free swelling culture promoted cell infiltration, sGAG accumulation, and enhanced the stiffness of constructs. The preculture of MSCs on aligned electrospun PCL scaffolds for 6 weeks in free swelling conditions followed by 6% dynamic tensile strain at 1 Hz for 3 hours/day for 4 weeks upregulated type I collagen, fibronectin, and lysyl oxidase mRNA levels (Baker et al., 2011). In addition, tensile loading enhanced

collagen deposition and the tensile modulus was increased from 27 MPa to 35 MPa but there was no enhancement of proteoglycan content. These results suggest that is it possible to recapitulate the structure and biomechanical environment of the meniscus to start approaching the functional properties for tissue engineered menisci but further optimization is still necessary to determine the optimal loading regimes and scaffold design to reproduce the mechanical properties of the meniscus and also the extracellular matrix structure and composition for tissue regeneration.

In order to prevent the long-term development of osteoarthritis, ideally clinical therapies following a meniscal tear would preserve the meniscal tissue to promote repair and restoration of a functional meniscal tissue. However, the difficulty of integrating torn meniscal tissue continues to be a challenge for long-term efficacy of meniscus repair strategies (McNulty and Guilak, 2008; Riera et al., 2011). An *in vitro* meniscal repair model system, using cylindrical porcine meniscal explants that have a central core removed with a biopsy punch and reinserted immediately to simulate a full thickness meniscal tear have been utilized to assess strategies to promote integrative meniscal repair (McNulty et al., 2010; McNulty et al., 2007). Physiologic concentrations of IL-1 decreased meniscal cell proliferation, and increased MMP activity, S-GAG release and NO production, and suppressed the integrative shear strength of repair (McNulty et al., 2010; McNulty et al., 2007; Riera et al., 2011). However, 1%, 10% and 26% dynamic strain at 1 Hz for 4 hours/day for 14 days antagonized the IL-1 inhibition of integrative meniscal repair (Figure 5) and suppressed IL-1 mediated increases in MMP activity, S-GAG release, and NO production (McNulty et al., 2010). Interestingly, 26% dynamic compression alone enhanced the integrative shear strength of repair. Therefore, joint loading following a meniscal tear may be beneficial to promote integrative tissue repair.

8. Conclusions

In summary, these studies provide significant *in vivo* and *in vitro* evidence that mechanical factors play an important role in the health, degeneration, and regeneration of the meniscus (Figure 6). It is evident that the process of mechanical signal transduction involves a complex sequence of mechanical and biochemical events. Through an array of experimental and theoretical studies, investigators have begun to directly measure the effects of joint loading on the meniscus, showing significant deformation and volume changes that occur under normal physiologic conditions. Using computational models and experimental pressure sensors, it is clear that the meniscus undergoes highly complex loading during the activities of daily living. Furthermore, theoretical and experimental models at the microscale level indicate that the cells in the meniscus are exposed to an environment of stress, strain, fluid pressure, fluid flow, and physicochemical factors that vary with time and site in the tissue. In 2D and 3D culture systems, these physical factors have been shown to have significant influences on meniscal cell responses, and single cell experiments have begun to provide new insights into the mechanisms by which mechanical and other biophysical signals are transduced. In this regard, a more thorough understanding of mechanobiologic responses of the meniscus will hopefully lead to new physical and/or pharmacologic therapies to prevent degeneration and enhance repair of the meniscus.

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References

Agarwal S, Long P, Gassner R, Piesco NP, Buckley MJ. Cyclic tensile strain suppresses catabolic effects of interleukin-1beta in fibrochondrocytes from the temporomandibular joint. Arthritis Rheum. 2001; 44:608–617. [PubMed: 11263775]

Anderson DR, Gershuni DH, Nakhostine M, Danzig LA. The effects of non-weight-bearing and limited motion on the tensile properties of the meniscus. Arthroscopy. 1993; 9:440–445. [PubMed: 8216576]

Andersson-Molina H, Karlsson H, Rockborn P. Arthroscopic partial and total meniscectomy: A long-term follow-up study with matched controls. Arthroscopy. 2002; 18:183–189. [PubMed: 11830813]

Arnoczky SP, Warren RF. The microvasculature of the meniscus and its response to injury. An experimental study in the dog. Am. J. Sports Med. 1983; 11:131–141. [PubMed: 6688156]

AufderHeide AC, Athanasiou KA. Mechanical stimulation toward tissue engineering of the knee meniscus. Ann. Biomed. Eng. 2004; 32:1161–1174. [PubMed: 15446512]

Aufderheide AC, Athanasiou KA. A direct compression stimulator for articular cartilage and meniscal explants. Ann. Biomed. Eng. 2006; 34:1463–1474. [PubMed: 16897420]

Baker BM, Shah RP, Huang AH, Mauck RL. Dynamic tensile loading improves the functional properties of mesenchymal stem cell-laden nanofiber-based fibrocartilage. Tissue Eng Part A. 2011; 17:1445–1455. [PubMed: 21247342]

Ballyns JJ, Bonassar LJ. Dynamic compressive loading of image-guided tissue engineered meniscal constructs. J. Biomech. 2011; 44:509–516. [PubMed: 20888562]

Bedi A, Kelly NH, Baad M, Fox AJ, Brophy RH, Warren RF, Maher SA. Dynamic contact mechanics of the medial meniscus as a function of radial tear, repair, and partial meniscectomy. J. Bone Joint Surg. Am. 2010; 92:1398–1408. [PubMed: 20516315]

Bland-Sutton, J. Ligaments: Their Nature and Morphology. H.K. Lewis; London: 1897.

Bray RC, Smith JA, Eng MK, Leonard CA, Sutherland CA, Salo PT. Vascular response of the meniscus to injury: effects of immobilization. J. Orthop. Res. 2001; 19:384–390. [PubMed: 11398850]

Burr DB, Frederickson RG, Pavlinch C, Sickles M, Burkart S. Intracast muscle stimulation prevents bone and cartilage deterioration in cast-immobilized rabbits. Clin Orthop Relat Res. 1984:264–278. [PubMed: 6478700]

Collier S, Ghosh P. Effects of transforming growth factor beta on proteoglycan synthesis by cell and explant cultures derived from the knee joint meniscus. Osteoarthritis Cartilage. 1995; 3:127–138. [PubMed: 7584318]

Deschner J, Wypasek E, Ferretti M, Rath B, Anghelina M, Agarwal S. Regulation of RANKL by biomechanical loading in fibrochondrocytes of meniscus. J. Biomech. 2006; 39:1796–1803. [PubMed: 16038916]

Djurasovic M, Aldridge JW, Grumbles R, Rosenwasser MP, Howell D, Ratcliffe A. Knee joint immobilization decreases aggrecan gene expression in the meniscus. Am. J. Sports Med. 1998; 26:460–466. [PubMed: 9617414]

Dowdy PA, Miniaci A, Arnoczky SP, Fowler PJ, Boughner DR. The effect of cast immobilization on meniscal healing. An experimental study in the dog. Am. J. Sports Med. 1995; 23:721–728. [PubMed: 8600741]

Eifler RL, Blough ER, Dehlin JM, Haut Donahue TL. Oscillatory fluid flow regulates glycosaminoglycan production via an intracellular calcium pathway in meniscal cells. J. Orthop. Res. 2006; 24:375–384. [PubMed: 16479571]

Fairbank TJ. Knee joint changes after meniscectomy. J. Bone Joint Surg. Br. 1948; 30B:664–670. [PubMed: 18894618]

Fermor B, Jeffcoat D, Hennerbichler A, Pisetsky DS, Weinberg JB, Guilak F. The effects of cyclic mechanical strain and tumor necrosis factor alpha on the response of cells of the meniscus. Osteoarthritis Cartilage. 2004; 12:956–962. [PubMed: 15564062]

Ferretti M, Madhavan S, Deschner J, Rath-Deschner B, Wypasek E, Agarwal S. Dynamic biophysical strain modulates proinflammatory gene induction in meniscal fibrochondrocytes. Am J Physiol Cell Physiol. 2006; 290:C1610–1615. [PubMed: 16452158]

Ferretti M, Srinivasan A, Deschner J, Gassner R, Baliko F, Piesco N, Salter R, Agarwal S. Anti-inflammatory effects of continuous passive motion on meniscal fibrocartilage. J. Orthop. Res. 2005; 23:1165–1171. [PubMed: 16140197]

Fink C, Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Guilak F. The effect of dynamic mechanical compression on nitric oxide production in the meniscus. Osteoarthritis Cartilage. 2001; 9:481–487. [PubMed: 11467897]

Fithian DC, Kelly MA, Mow VC. Material properties and structure-function relationships in the menisci. Clin Orthop Relat Res. 1990:19–31. [PubMed: 2406069]

Freutel M, Seitz AM, Galbusera F, Bornstedt A, Rasche V, Knothe Tate ML, Ignatius A, Durselen L. Medial meniscal displacement and strain in three dimensions under compressive loads: MR assessment. J. Magn. Reson. Imaging. 2014; 40:1181–1188. [PubMed: 24323799]

Furumatsu T, Kanazawa T, Miyake Y, Kubota S, Takigawa M, Ozaki T. Mechanical stretch increases Smad3-dependent CCN2 expression in inner meniscus cells. J. Orthop. Res. 2012; 30:1738–1745. [PubMed: 22576977]

Guilak F, Butler DL, Goldstein SA, Baaijens FP. Biomechanics and mechanobiology in functional tissue engineering. J. Biomech. 2014; 47:1933–1940. [PubMed: 24818797]

Guilak F, Mow VC. The mechanical environment of the chondrocyte: a biphasic finite element model of cell-matrix interactions in articular cartilage. J. Biomech. 2000; 33:1663–1673. [PubMed: 11006391]

Gupta T, Haut Donahue TL. Role of cell location and morphology in the mechanical environment around meniscal cells. Acta Biomater. 2006; 2:483–492. [PubMed: 16860617]

Gupta T, Zielinska B, McHenry J, Kadmiel M, Haut Donahue TL. IL-1 and iNOS gene expression and NO synthesis in the superior region of meniscal explants are dependent on the magnitude of compressive strains. Osteoarthritis Cartilage. 2008; 16:1213–1219. [PubMed: 18439846]

Hall M, Wrigley TV, Metcalf BR, Cicuttini FM, Wang Y, Hinman RS, Dempsey AR, Mills PM, Lloyd DG, Bennell KL. Do Moments and Strength Predict Cartilage Changes following Partial Meniscectomy? Med. Sci. Sports Exerc. 2014

Halonen KS, Mononen ME, Jurvelin JS, Toyras J, Salo J, Korhonen RK. Deformation of articular cartilage during static loading of a knee joint--experimental and finite element analysis. J. Biomech. 2014; 47:2467–2474. [PubMed: 24813824]

Han WM, Heo SJ, Driscoll TP, Boggs ME, Duncan RL, Mauck RL, Elliott DM. Impact of cellular microenvironment and mechanical perturbation on calcium signalling in meniscus fibrochondrocytes. Eur Cell Mater. 2014; 27:321–331. [PubMed: 24908425]

Han WM, Heo SJ, Driscoll TP, Smith LJ, Mauck RL, Elliott DM. Macro- to microscale strain transfer in fibrous tissues is heterogeneous and tissue-specific. Biophys. J. 2013; 105:807–817. [PubMed: 23931328]

Hellio Le Graverand MP, Ou Y, Schield-Yee T, Barclay L, Hart D, Natsume T, Rattner JB. The cells of the rabbit meniscus: their arrangement, interrelationship, morphological variations and cytoarchitecture. J. Anat. 2001; 198:525–535. [PubMed: 11430692]

Hennerbichler A, Fermor B, Hennerbichler D, Weinberg JB, Guilak F. Regional differences in prostaglandin E2 and nitric oxide production in the knee meniscus in response to dynamic compression. Biochem. Biophys. Res. Commun. 2007; 358:1047–1053. [PubMed: 17517372]

Hoser C, Fink C, Brown C, Reichkendler M, Hackl W, Bartlett J. Long-term results of arthroscopic partial lateral meniscectomy in knees without associated damage. J. Bone Joint Surg. Br. 2001; 83-B:513–516. [PubMed: 11380121]

Huey DJ, Athanasiou KA. Tension-compression loading with chemical stimulation results in additive increases to functional properties of anatomic meniscal constructs. PLoS One. 2011; 6:e27857. [PubMed: 22114714]

Imler SM, Doshi AN, Levenston ME. Combined effects of growth factors and static mechanical compression on meniscus explant biosynthesis. Osteoarthritis Cartilage. 2004; 12:736–744. [PubMed: 15325640]

Kanazawa T, Furumatsu T, Hachioji M, Oohashi T, Ninomiya Y, Ozaki T. Mechanical stretch enhances COL2A1 expression on chromatin by inducing SOX9 nuclear translocalization in inner meniscus cells. J. Orthop. Res. 2012; 30:468–474. [PubMed: 21853455]

Kawahara Y, Uetani M, Fuchi K, Eguchi H, Hayashi K. MR assessment of movement and morphologic change in the menisci during knee flexion. Acta Radiol. 1999; 40:610–614. [PubMed: 10598848]

Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Volume changes in the menisci and articular cartilage of runners: an in vivo investigation based on 3-D magnetic resonance imaging. Am. J. Sports Med. 2006; 34:832–836. [PubMed: 16436539]

Killian ML, Haut RC, Haut Donahue TL. Acute cell viability and nitric oxide release in lateral menisci following closed-joint knee injury in a lapine model of post-traumatic osteoarthritis. BMC Musculoskelet Disord. 2014; 15:297. [PubMed: 25192881]

Killian ML, Zielinska B, Gupta T, Haut Donahue TL. In vitro inhibition of compression-induced catabolic gene expression in meniscal explants following treatment with IL-1 receptor antagonist. J. Orthop. Sci. 2011; 16:212–220. [PubMed: 21331553]

Kisiday JD, Vanderploeg EJ, McIlwraith CW, Grodzinsky AJ, Frisbie DD. Mechanical injury of explants from the articulating surface of the inner meniscus. Arch. Biochem. Biophys. 2010; 494:138–144. [PubMed: 19944061]

Klein L, Heiple KG, Torzilli PA, Goldberg VM, Burstein AH. Prevention of ligament and meniscus atrophy by active joint motion in a non-weight-bearing model. J. Orthop. Res. 1989; 7:80–85. [PubMed: 2908915]

Klein L, Player JS, Heiple KG, Bahniuk E, Goldberg VM. Isotopic evidence for resorption of soft tissues and bone in immobilized dogs. J. Bone Joint Surg. Am. 1982; 64:225–230. [PubMed: 7056777]

Lai JH, Levenston ME. Meniscus and cartilage exhibit distinct intra-tissue strain distributions under unconfined compression. Osteoarthritis Cartilage. 2010; 18:1291–1299. [PubMed: 20633686]

LeRoux MA, Setton LA. Experimental and biphasic FEM determinations of the material properties and hydraulic permeability of the meniscus in tension. J. Biomech. Eng. 2002; 124:315–321. [PubMed: 12071267]

Liu C, Abedian R, Meister R, Haasper C, Hurschler C, Krettek C, von Lewinski G, Jagodzinski M. Influence of perfusion and compression on the proliferation and differentiation of bone mesenchymal stromal cells seeded on polyurethane scaffolds. Biomaterials. 2012; 33:1052–1064. [PubMed: 22056755]

Makris EA, Hadidi P, Athanasiou KA. The knee meniscus: structure-function, pathophysiology, current repair techniques, and prospects for regeneration. Biomaterials. 2011; 32:7411–7431. [PubMed: 21764438]

Martinez H, Brackmann C, Enejder A, Gatenholm P. Mechanical stimulation of fibroblasts in micro-channeled bacterial cellulose scaffolds enhances production of oriented collagen fibers. J Biomed Mater Res A. 2012; 100:948–957. [PubMed: 22275210]

McDevitt, CA.; Li, H.; Zaramo, C.; Prajapati, R. The isolation of a cell-pericellular matrix complex from meniscus fibrocartilage: The fibrochondron. Transactions of the Orthopaedic Research Society; 2001. Poster #0384

McDevitt CA, Webber RJ. The ultrastructure and biochemistry of meniscal cartilage. Clin Orthop Relat Res. 1990:8–18. [PubMed: 2406077]

McHenry JA, Zielinska B, Donahue TL. Proteoglycan breakdown of meniscal explants following dynamic compression using a novel bioreactor. Ann. Biomed. Eng. 2006; 34:1758–1766. [PubMed: 17031596]

McNulty AL, Estes BT, Wilusz RE, Weinberg JB, Guilak F. Dynamic loading enhances integrative meniscal repair in the presence of interleukin-1. Osteoarthritis Cartilage. 2010; 18:830–838. [PubMed: 20202487]

McNulty AL, Guilak F. Integrative repair of the meniscus: lessons from in vitro studies. Biorheology. 2008; 45:487–500. [PubMed: 18836248]

McNulty AL, Moutos FT, Weinberg JB, Guilak F. Enhanced integrative repair of the porcine meniscus in vitro by inhibition of interleukin-1 or tumor necrosis factor alpha. Arthritis Rheum. 2007; 56:3033–3042. [PubMed: 17729298]

McNulty AL, Rothfusz NE, Leddy HA, Guilak F. Synovial fluid concentrations and relative potency of interleukin-1 alpha and beta in cartilage and meniscus degradation. J. Orthop. Res. 2013; 31:1039–1045. [PubMed: 23483596]

Meakin JR, Shrive NG, Frank CB, Hart DA. Finite element analysis of the meniscus: the influence of geometry and material properties on its behaviour. Knee. 2003; 10:33–41. [PubMed: 12649024]

Mikic B, Johnson TL, Chhabra AB, Schalet BJ, Wong M, Hunziker EB. Differential effects of embryonic immobilization on the development of fibrocartilaginous skeletal elements. J. Rehabil. Res. Dev. 2000; 37:127–133. [PubMed: 10850818]

Nathan AS, Baker BM, Nerurkar NL, Mauck RL. Mechano-topographic modulation of stem cell nuclear shape on nanofibrous scaffolds. Acta Biomater. 2011; 7:57–66. [PubMed: 20709198]

Natsu-Ume T, Majima T, Reno C, Shrive NG, Frank CB, Hart DA. Menisci of the rabbit knee require mechanical loading to maintain homeostasis: cyclic hydrostatic compression in vitro prevents derepression of catabolic genes. J. Orthop. Sci. 2005; 10:396–405. [PubMed: 16075173]

Nerurkar NL, Sen S, Baker BM, Elliott DM, Mauck RL. Dynamic culture enhances stem cell infiltration and modulates extracellular matrix production on aligned electrospun nanofibrous scaffolds. Acta Biomater. 2011; 7:485–491. [PubMed: 20728589]

Neves AA, Medcalf N, Brindle KM. Tissue engineering of meniscal cartilage using perfusion culture. Ann. N. Y. Acad. Sci. 2002; 961:352–355. [PubMed: 12081938]

Nishimuta JF, Levenston ME. Response of cartilage and meniscus tissue explants to in vitro compressive overload. Osteoarthritis Cartilage. 2012; 20:422–429. [PubMed: 22289896]

Ochi M, Kanda T, Sumen Y, Ikuta Y. Changes in the permeability and histologic findings of rabbit menisci after immobilization. Clin Orthop Relat Res. 1997:305–315. [PubMed: 9005928]

Pangborn CA, Athanasiou KA. Effects of growth factors on meniscal fibrochondrocytes. Tissue Eng. 2005; 11:1141–1148. [PubMed: 16144450]

Pedrini-Mille A, Pedrini VA, Maynard JA, Vailas AC. Response of immature chicken meniscus to strenuous exercise: biochemical studies of proteoglycan and collagen. J. Orthop. Res. 1988; 6:196–204. [PubMed: 3125312]

Petri M, Ufer K, Toma I, Becher C, Liodakis E, Brand S, Haas P, Liu C, Richter B, Haasper C, von Lewinski G, Jagodzinski M. Effects of perfusion and cyclic compression on in vitro tissue engineered meniscus implants. Knee Surg. Sports Traumatol. Arthrosc. 2012; 20:223–231. [PubMed: 21750950]

Puetzer JL, Ballyns JJ, Bonassar LJ. The effect of the duration of mechanical stimulation and post-stimulation culture on the structure and properties of dynamically compressed tissue-engineered menisci. Tissue Eng Part A. 2012; 18:1365–1375. [PubMed: 22429287]

Riera KM, Rothfusz NE, Wilusz RE, Weinberg JB, Guilak F, McNulty AL. Interleukin-1, tumor necrosis factor-alpha, and transforming growth factor-beta 1 and integrative meniscal repair: influences on meniscal cell proliferation and migration. Arthritis Res Ther. 2011; 13:R187. [PubMed: 22087734]

Roos EM, Ostenberg A, Roos H, Ekdahl C, Lohmander LS. Long-term outcome of meniscectomy: symptoms, function, and performance tests in patients with or without radiographic osteoarthritis compared to matched controls. Osteoarthritis Cartilage. 2001; 9:316–324. [PubMed: 11399095]

Sanchez-Adams J, Athanasiou KA. Biomechanics of meniscus cells: regional variation and comparison to articular chondrocytes and ligament cells. Biomech Model Mechanobiol. 2012; 11:1047–1056. [PubMed: 22231673]

Sanchez-Adams J, Wilusz RE, Guilak F. Atomic force microscopy reveals regional variations in the micromechanical properties of the pericellular and extracellular matrices of the meniscus. J. Orthop. Res. 2013; 31:1218–1225. [PubMed: 23568545]

Scott GA, Jolly BL, Henning CE. Combined posterior incision and arthroscopic intra-articular repair of the meniscus. An examination of factors affecting healing. J. Bone Joint Surg. Am. 1986; 68:847–861. [PubMed: 3755440]

Setton LA, Guilak F, Hsu EW, Vail TP. Biomechanical factors in tissue engineered meniscal repair. Clin Orthop Relat Res. 1999:S254–272. [PubMed: 10546651]

Shin SJ, Fermor B, Weinberg JB, Pisetsky DS, Guilak F. Regulation of matrix turnover in meniscal explants: role of mechanical stress, interleukin-1, and nitric oxide. J Appl Physiol (1985). 2003; 95:308–313. [PubMed: 12665533]

Spilker RL, Donzelli PS, Mow VC. A transversely isotropic biphasic finite element model of the meniscus. J. Biomech. 1992; 25:1027–1045. [PubMed: 1517263]

Stehling C, Luke A, Stahl R, Baum T, Joseph G, Pan J, Link TM. Meniscal T1rho and T2 measured with 3.0T MRI increases directly after running a marathon. Skeletal Radiol. 2011; 40:725–735. [PubMed: 21052658]

Suzuki T, Toyoda T, Suzuki H, Hisamori N, Matsumoto H, Toyama Y. Hydrostatic pressure modulates mRNA expressions for matrix proteins in human meniscal cells. Biorheology. 2006; 43:611–622. [PubMed: 17047280]

Tienen TG, Buma P, Scholten JG, van Kampen A, Veth RP, Verdonschot N. Displacement of the medial meniscus within the passive motion characteristics of the human knee joint: an RSA study in human cadaver knees. Knee Surg. Sports Traumatol. Arthrosc. 2005; 13:287–292. [PubMed: 15309283]

Upton ML, Chen J, Guilak F, Setton LA. Differential effects of static and dynamic compression on meniscal cell gene expression. J. Orthop. Res. 2003; 21:963–969. [PubMed: 14554206]

Upton ML, Gilchrist CL, Guilak F, Setton LA. Transfer of macroscale tissue strain to microscale cell regions in the deformed meniscus. Biophys. J. 2008; 95:2116–2124. [PubMed: 18487290]

Upton ML, Guilak F, Laursen TA, Setton LA. Finite element modeling predictions of region-specific cell-matrix mechanics in the meniscus. Biomech Model Mechanobiol. 2006; 5:140–149. [PubMed: 16520958]

Upton ML, Hennerbichler A, Fermor B, Guilak F, Weinberg JB, Setton LA. Biaxial strain effects on cells from the inner and outer regions of the meniscus. Connect. Tissue Res. 2006; 47:207–214. [PubMed: 16987752]

Vailas AC, Zernicke RF, Matsuda J, Curwin S, Durivage J. Adaptation of rat knee meniscus to prolonged exercise. J Appl Physiol. 1986; 60:1031–1034. [PubMed: 3957819]

Vanderploeg EJ, Imler SM, Brodkin KR, Garcia AJ, Levenston ME. Oscillatory tension differentially modulates matrix metabolism and cytoskeletal organization in chondrocytes and fibrochondrocytes. J. Biomech. 2004; 37:1941–1952. [PubMed: 15519602]

Vedi V, Williams A, Tennant SJ, Spouse E, Hunt DM, Gedroyc WM. Meniscal movement. An in-vivo study using dynamic MRI. J. Bone Joint Surg. Br. 1999; 81:37–41. [PubMed: 10067999]

Verdonk PC, Forsyth RG, Wang J, Almqvist KF, Verdonk R, Veys EM, Verbruggen G. Characterisation of human knee meniscus cell phenotype. Osteoarthritis Cartilage. 2005; 13:548–560. [PubMed: 15979007]

Videman T, Eronen I, Friman C, Langenskiold A. Glycosaminoglycan metabolism of the medial meniscus, the medial collateral ligament and the hip joint capsule in experimental osteoarthritis caused by immobilization of the rabbit knee. Acta Orthop. Scand. 1979; 50:465–470. [PubMed: 582870]

Walker PS, Erkman MJ. The role of the menisci in force transmission across the knee. Clin Orthop Relat Res. 1975:184–192. [PubMed: 1173360]

Wilusz RE, Sanchez-Adams J, Guilak F. The structure and function of the pericellular matrix of articular cartilage. Matrix Biol. 2014; 39C:25–32. [PubMed: 25172825]

Yao J, Snibbe J, Maloney M, Lerner AL. Stresses and strains in the medial meniscus of an ACL deficient knee under anterior loading: a finite element analysis with image-based experimental validation. J. Biomech. Eng. 2006; 128:135–141. [PubMed: 16532627]

Zielinska B, Killian M, Kadmiel M, Nelsen M, Haut Donahue TL. Meniscal tissue explants response depends on level of dynamic compressive strain. Osteoarthritis Cartilage. 2009; 17:754–760. [PubMed: 19121588]

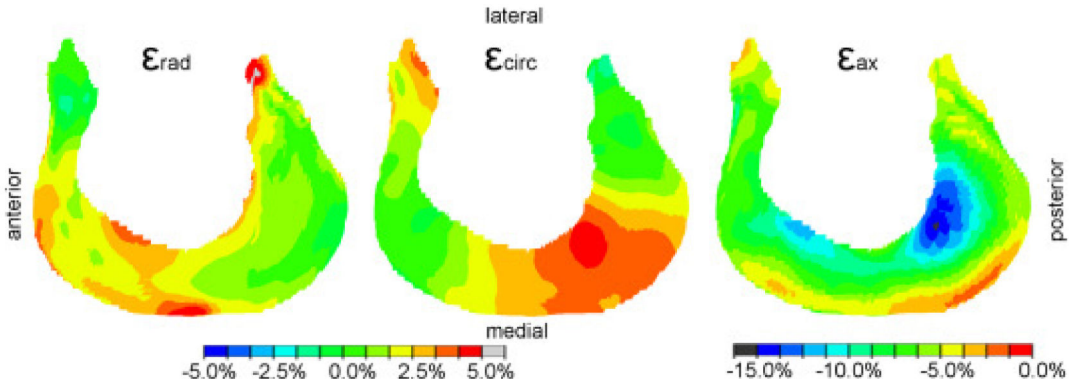

Figure 1. High-resolution MRI was combined with image registration to measure the displacement and strain of the meniscus and its attachments under compression

Local strain of one representative meniscus and its attachments (view from proximal) in radial (ε_{rad}), circumferential (ε_{circ}), and axial (ε_{ax}) direction under 100% body weight (positive values indicate tension, negative values indicate compression). The meniscus and its attachments showed low average radial or circumferential stretch (less than 1%), but axial strains of nearly 12% during physiologically relevant loading of the knee. Reprinted with permission from (Freutel et al., 2014).

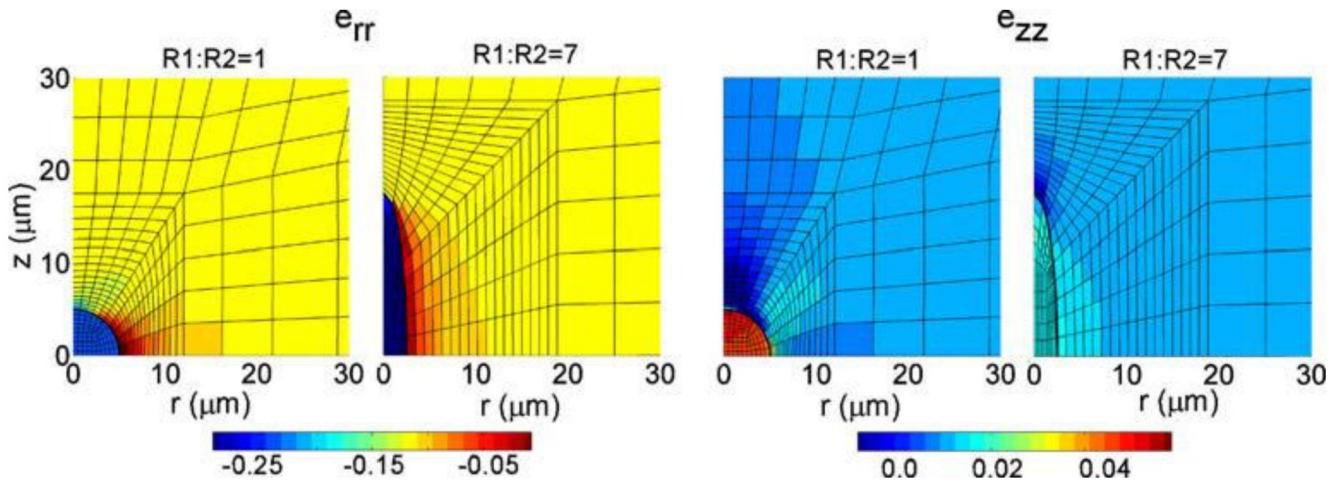

Figure 2. A finite element model of cell-matrix interactions was developed to investigate the mechanical environment of inner and outer meniscus cells with varying geometries

Pseudocolor plots depicting effects of cell geometry on cell mechanics in an isotropic extracellular matrix in response to statically applied biaxial strain (e_{zz} = 0.01, e_{rr} = −0.1). Shown are the radial (e_{rr}) and axial (e_{zz}) Eulerian finite strain components at equilibrium (t > 10^4 s) plotted on spatial coordinates. Compressive transverse strains in meniscus cells of both regions were approximately two to three-fold higher than those in the extracellular matrix and exhibited a modest association with cell aspect ratio. Reprinted with permission from (Upton et al., 2006).

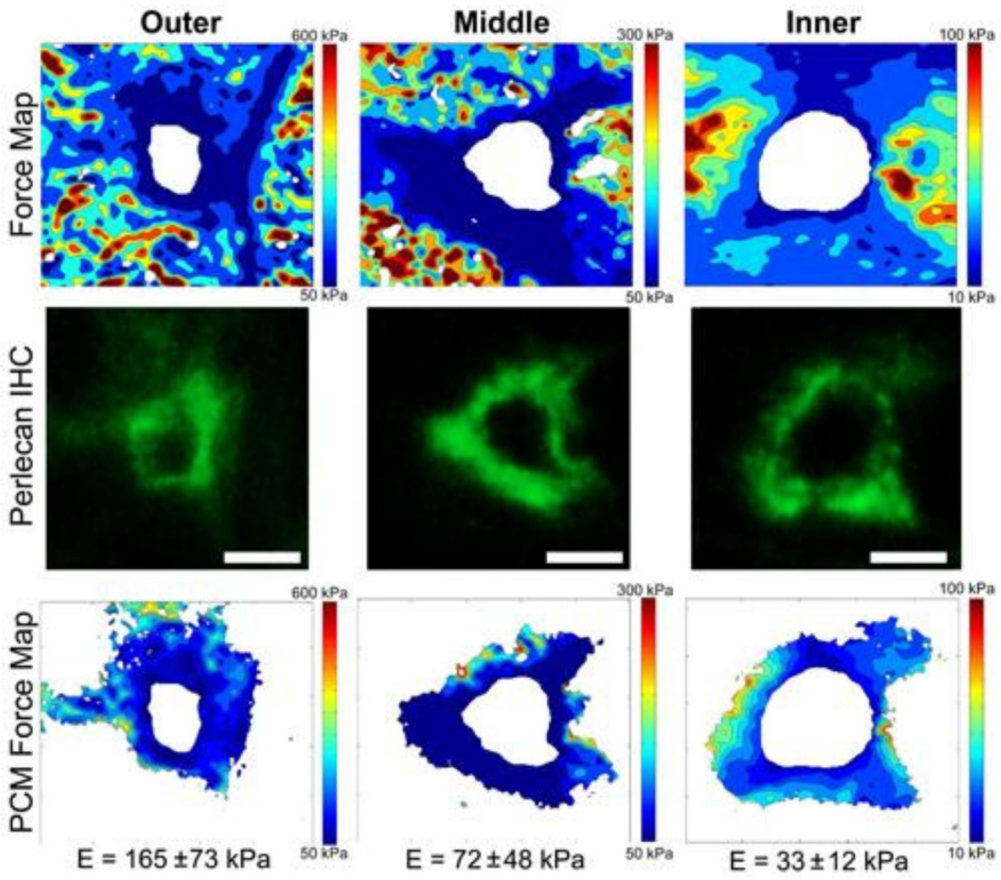

Figure 3. Immunofluorescence-guided atomic force microscopy (AFM) was used to spatially map the mechanical properties of the pericellular matrix (PCM) of the meniscus

Force maps (20 × 20 μm) of pericellular sites within each meniscus region (top row) were integrated with fluorescent perlecan staining (middle row) to define PCM boundaries. The resulting PCM force maps were analyzed to yield an average elastic modulus (bottom row). The elastic modulus of the meniscus pericellular matrix was significantly higher in the outer region than the inner region, whereas extracellular matrix moduli were consistently higher than region-matched pericellular matrices in both the outer and inner regions. Adapted from (Sanchez-Adams et al., 2013) and (Wilusz et al., 2014).

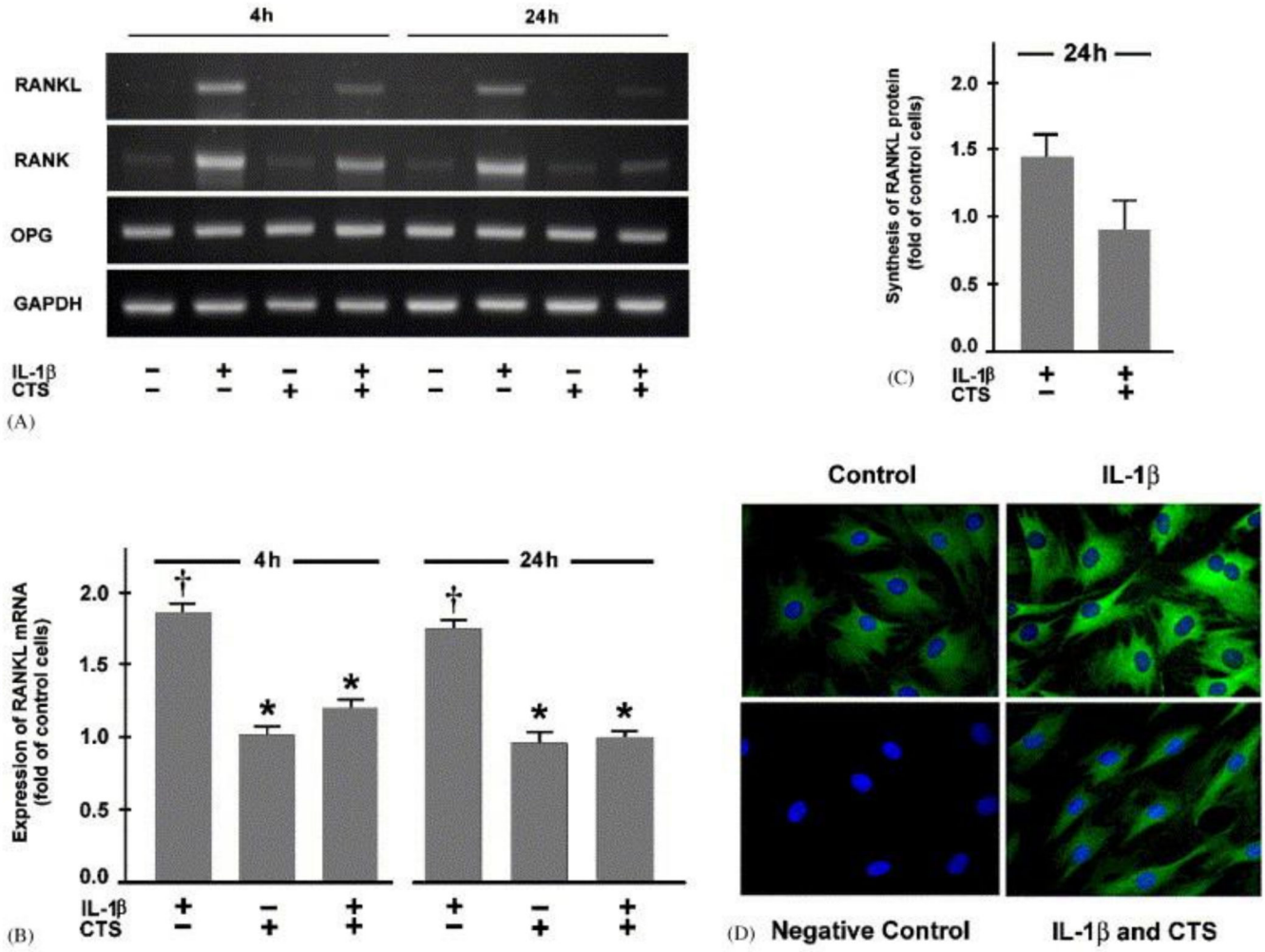

Figure 4. Cyclic tensile strain (CTS) can exhibit an anti-inflammatory effect on meniscal cells

Fibrochondrocytes from meniscus were subjected to CTS at a magnitude of 20% and 0.05 Hz in the presence and absence of 1 ng/mL interleukin (IL)-1β. (A) mRNA expression for RANKL, RANK, and OPG at 4 and 24 h, as determined by semiquantitative RT-PCR. (B) Quantitative assessment of RANKL mRNA at 4 and 24 h, as determined by real-time PCR. Results are shown as means ± S.E.M., n=6, † significantly ($p<0.05$) different from control cells and stretched cells in the presence or absence of IL-1β. * significantly ($p<0.05$) different from unstretched IL-1β-treated cells. (C) Protein synthesis of RANKL at 24 h, as analyzed by Western blot and subsequent semiquantitative densitometry. Results are shown as means ± S.E.M., n=3. (D) Protein synthesis of RANK at 24 h, as analyzed by immunofluorescence. IL-1β treatment caused the upregulation of RANK and RANKL but did not alter OPG expression. Conversely, cyclic tensile strain suppressed the IL-1 mediated upregulation of RANK and RANKL. Reprinted with permission from (Deschner et al., 2006).

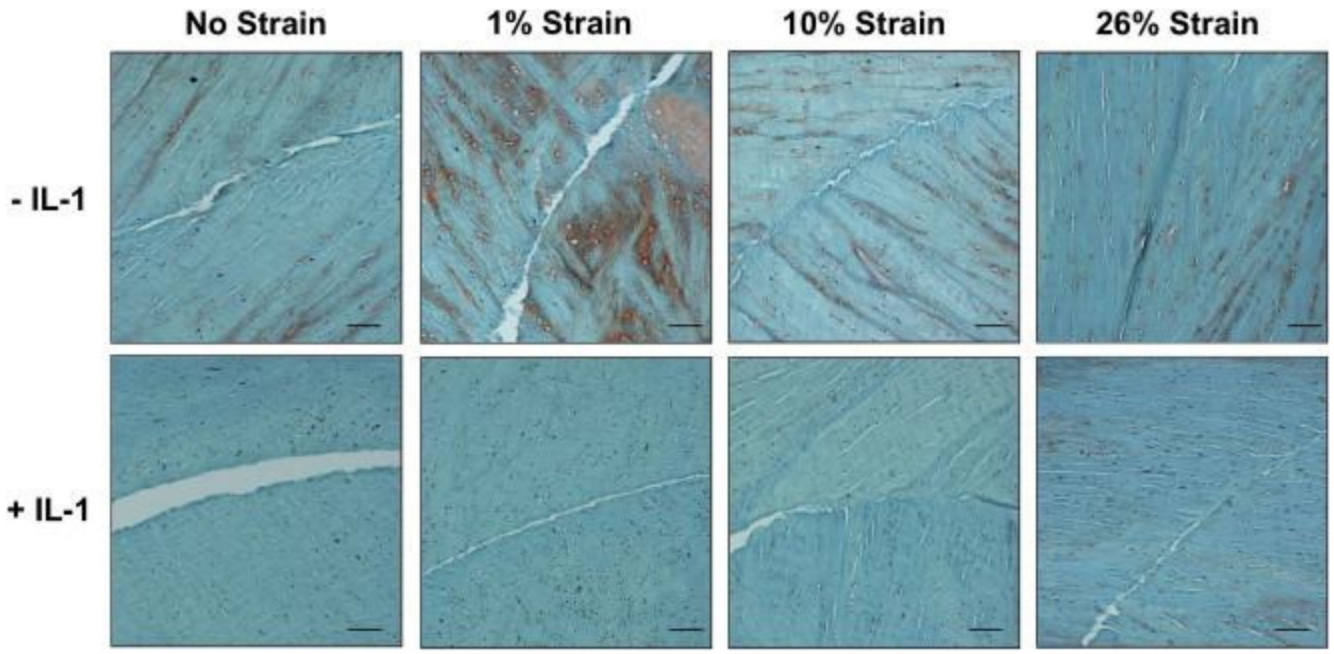

Figure 5. Dynamic mechanical loading enhances meniscal repair *in vitro*

Histological images of paraffin embedded meniscal repair model explants stained with Hematoxylin to identify the cell nuclei (black), fast green (green) to identify the collagen fibers, and Safranin O (red) to stain proteoglycans. Samples were incubated with no IL-1 or 100 pg/mL IL-1 and subjected to 0%, 1%, 10%, or 26% strain. IL-1 treatment prevented tissue repair and resulted in loss of proteoglycan staining in the meniscal extracellular matrix, whereas dynamic compression increased interfacial tissue repair in meniscal explants treated with IL-1. Scale bar is equal to 100 μm. Reprinted from (McNulty et al., 2010).

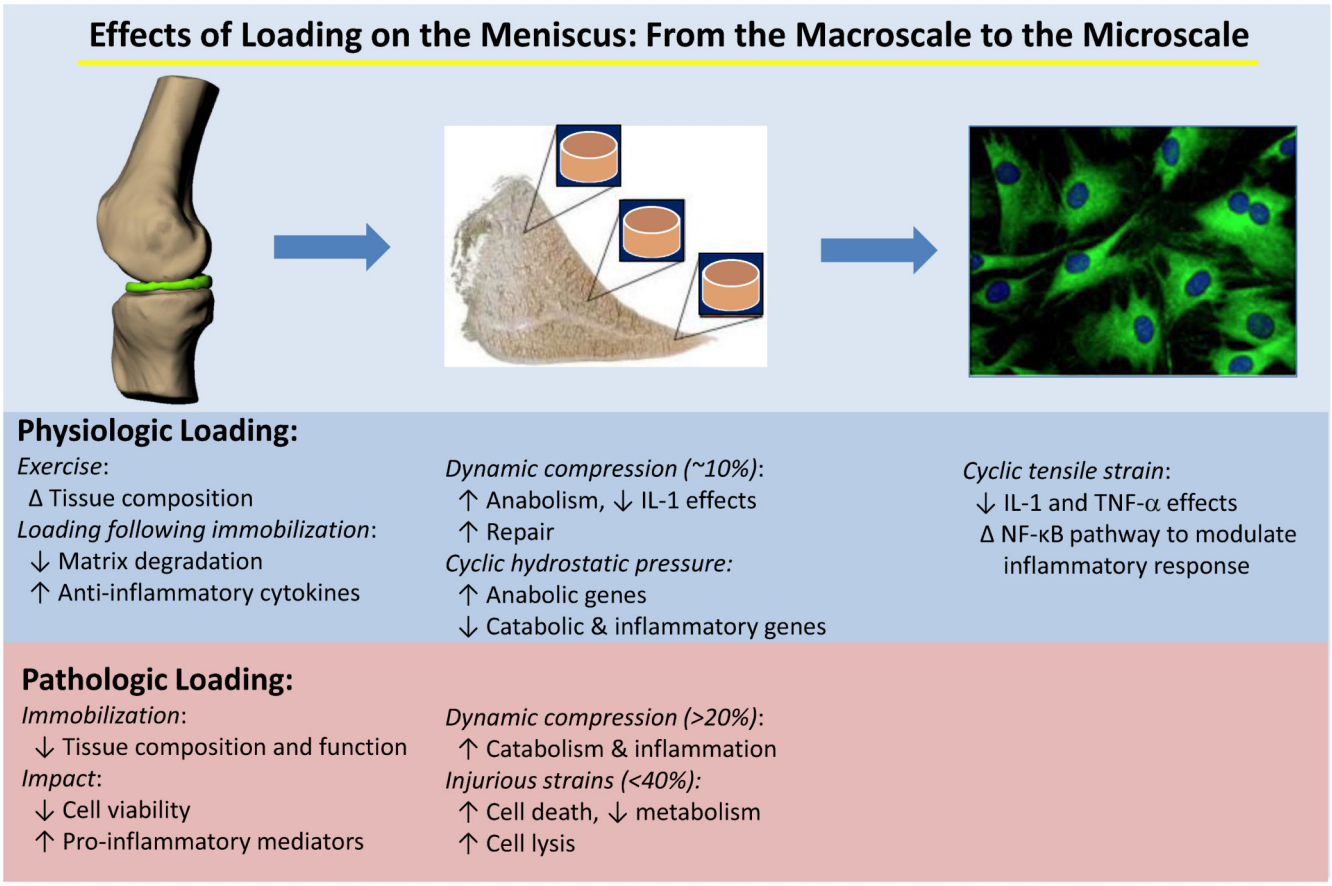

Figure 6. Macroscale to microscale studies have elucidated the effects of loading on the meniscus

Numerous studies have assessed the effects of physiologic and pathologic loading regimes on the meniscus at a range of different scales, including joint level studies, explant studies, and experiments using isolated meniscal cells. The meniscal cell image is reprinted with permission from (Deschner et al., 2006).