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Review

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Effects of Inflammation on Multiscale Biomechanical Properties of Cartilaginous Cells and Tissues

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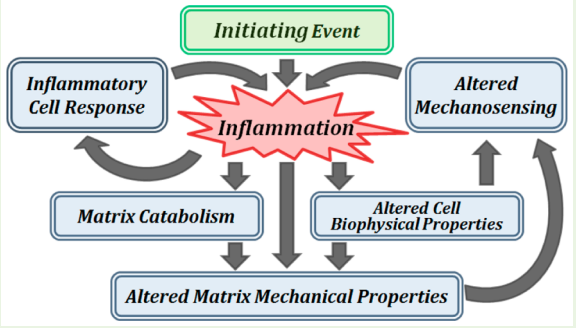
ABSTRACT: Cells within cartilaginous tissues are mechanosensitive and thus require mechanical loading for regulation of tissue homeostasis and metabolism. Mechanical loading plays critical roles in cell differentiation, proliferation, biosynthesis, and homeostasis. Inflammation is an important event occurring during multiple processes, such as aging, injury, and disease. Inflammation has significant effects on biological processes as well as mechanical function of cells and tissues. These effects are highly dependent on cell/tissue type, timing, and magnitude. In this review, we summarize key findings pertaining to effects of inflammation on multiscale mechanical properties at subcellular, cellular, and tissue level in cartilaginous tissues, including alterations in mechanotransduction and mechanosensitivity. The emphasis is on articular cartilage and the intervertebral disc, which are impacted by inflammatory insults during degenerative conditions such as osteoarthritis, joint pain, and back pain. To recapitulate the pro-inflammatory cascades that occur in vivo, different inflammatory stimuli have been used for in vitro and in situ studies, including tumor necrosis factor (TNF), various interleukins (IL), and lipopolysaccharide (LPS). Therefore, this review will focus on the effects of these stimuli because they are the best studied pro-inflammatory cytokines in cartilaginous tissues. Understanding the current state of the field of inflammation and cell/tissue biomechanics may potentially identify future directions for novel and translational therapeutics with multiscale biomechanical considerations.



KEYWORDS: *multiscale biomechanics, cytokines, cartilage, intervertebral disc, mechanobiology, mechanotransduction*

1. INTRODUCTION

Cartilaginous tissues are a type of connective tissue in the musculoskeletal system that functions as a load bearing material and provides joint flexibility and stability during body movement. Cartilaginous tissue is characterized by an extracellular matrix that is rich in proteoglycan and collagen and has high water content. Articular cartilage and the intervertebral disc (IVD) are considered two major cartilaginous tissues in the body. Cartilage and IVD tissue are exposed to loading throughout life. Articular cartilage covers the ends of long bones and functions to bear load and to provide a frictionless sliding during joint movement. Knee cartilage is compressed by ∼3−10% of its overall thickness following various physical activities, such as walking, cycling, running, and knee bending.[1,2] Under normal loading, talar cartilage is compressed by 5−35%, with 42% of the contact area having compressive strain higher than 15%.[3] The IVD has a heterogeneous structure with distinct regions: the central nucleus pulposus (NP), outer annulus fibrosus (AF), and cartilage endplate (CEP). IVD functions as a cushion to absorb load to protect the vertebral body during body motion, such as bending, twisting, and jumping.[4,5] The AF experiences a combination of compressive, tensile and shear stresses during weight-bearing and joint motions.[6−10] The NP has also been shown to translate and deform with different loading conditions in experimental studies.[11−13]

Cells within cartilaginous tissues are "mechanosensitive" and thus respond to mechanical loading. Cells regulate tissue homeostasis and are responsible for synthesis and degradation of matrix proteins. Mechanical loading plays critical roles in cell differentiation, proliferation, biosynthesis, and homeostasis. Overloading or underloading could lead to cell apoptosis, tissue matrix degradation, and increased expression of pro-inflammatory cytokines,[14−17] whereas moderate levels of mechanical stimulation maintain normal tissue function and have anti-inflammatory effects.[18−20]

Inflammation is an important signaling process that occurs in multiple conditions, such as aging, injury, disease, and in response to mechanical loading. These conditions can all serve as primary initiating events of abnormal production of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-

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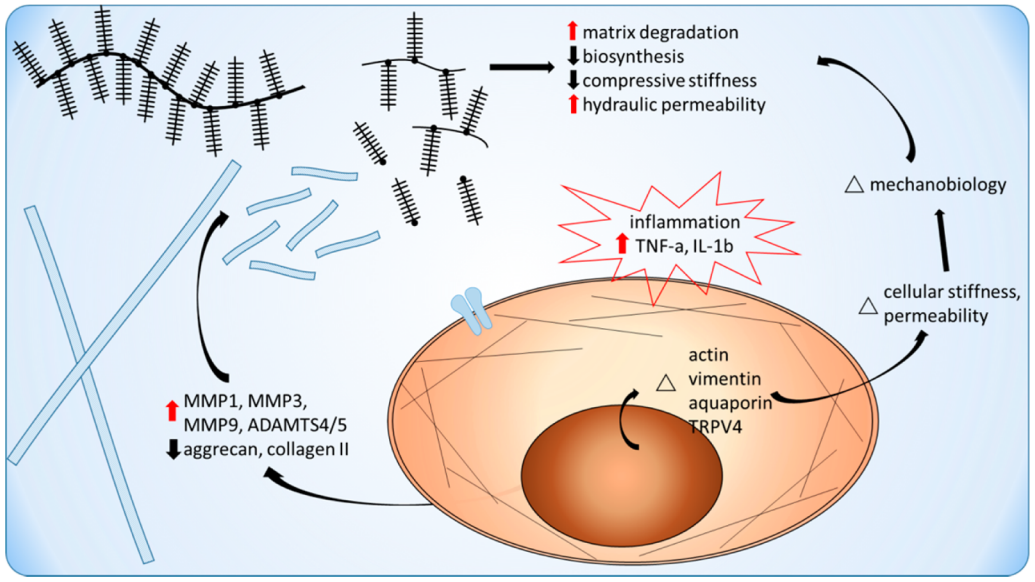

Figure 1. Schematic of integrated effects of pro-inflammatory cytokines on cell and tissue mechanical function and mechanotransduction in cartilaginous tissues.

α), interleukin-1α/β (IL-1α/β), IL-6, IL-8, and others. Of these cytokines, TNF-α and IL-1β are the best studied pro-inflammatory cytokines because they play a prominent role in tissue degeneration of IVD (reviewed in ref 21) and cartilage (reviewed in ref 22 and 23). In the IVD, these cytokines trigger a range of changes in disc cell phenotypes, including autophagy, senescence, and apoptosis.[24−26] It has been shown that TNF-α and IL-1β levels are elevated in degenerate discs and their expression increased with disease severity.[27,28] TNF-α and IL-1β induce upregulation of catabolic mediators such as MMP-1, -2, -3, -13, -14, ADAMTS-4/5 and suppress the expression of important matrix genes.[27−30] With regard to articular cartilage, levels of TNF-α and IL-1β in joint fluids were elevated acutely following traumatic joint injury compared to uninjured controls,[31] and remain elevated in the joints of patients with OA. These cytokines are thought to be involved in the pathophysiology of OA[32,33] by stimulating the production of matrix degrading enzymes and other inflammatory cytokines and mediators from chondrocytes and noncartilaginous cells present in the joint (e.g., synovium).[34−36]

Aging in cartilaginous tissues is characterized by many changes that are similar to those seen in degenerative disease conditions making it challenging to delineate where "healthy" aging and disease interface. Inflammation, which plays a large part in degenerative disease conditions, has also long been associated with aging. The idea of "inflammaging" as first coined by Franceschi et al. in 2000 has shown that systemic levels of inflammatory cytokines and markers increase with age.[37,38] On a local tissue level, cartilaginous tissues also exhibit this age dependent increase in inflammatory cytokines and markers (reviewed in ref 39). For example, aging in the IVD has indeed been shown to be associated with increased levels of TNF and TNF-R expression in NP cells.[40] Additional age-associated changes within the IVD may also contribute to an increasingly inflammatory environment in the disc with age. These changes include cell senescence and pro-inflammatory senescent phenotype, increased levels of reactive oxygen species (ROS), accumulation of advanced glycation end products (AGEs), and increased levels of matrix degrading enzymes.[39] Furthermore, studies have shown that NF-kB, a key transcription factor in inflammatory signaling pathways, has increased activity with age.[41] Articular cartilage presents many of the same age associated changes as seen in the IVD. Cell senesce and pro-inflammatory senescent phenotype, increased presence of matrix degrading enzymes, accumulation of AGEs and ROS which contribute to oxidative stress and damage all promote an inflammatory environment associated with aging in cartilage.[42−44]

During inflammation and degeneration processes in cartilaginous tissues, there is a shift in extracellular matrix (ECM) homeostasis away from anabolic metabolism toward more catabolic processes. As a result, the biological and biomechanical properties of tissues are altered. The biological changes in articular cartilage and IVD include loss of matrix proteins and altered water content.[45−51] The resulting biomechanical alterations in articular cartilage and IVD, however, have different functional manifestation. As cartilage undergoes degeneration in osteoarthritis, articular surfaces become fibrillated and roughened,[52] leading to increased surface interaction between articulating cartilage surfaces, decreased shear stiffness, and increased deformation near the surface.[53] Compared to normal discs, degenerated discs have a smaller NP area with a lower hydrostatic pressure and a wider posterior annulus with higher stress peaks.[4] Structural changes in cartilaginous tissues with degeneration may lead to alterations in load transfer, which may cause pain and lead to further matrix disruption and deterioration.[4,11,21,50,53−55]

The differential effects of inflammation on cellular processes as well as mechanical function are highly dependent on cell/tissue type, timing, and magnitude. In this review, we summarize key findings pertaining to inflammation, degeneration, and multiscale mechanical properties at the subcellular, cellular, and matrix level in cartilaginous tissues (Figure 1), with emphasis on articular cartilage and NP and AF regions of the IVD. Some inflammatory effects in cartilaginous tissues are similar in articular cartilage and IVD while others are different. This review aims to compare similaries and contrast differences between the two cartilaginous tissue types, to reflect on the functional uniqueness of each tissue/cell type. To recapitulate the pro-inflammatory cascades that occur in vivo, different

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Table 1. Changes in Tissue Level Biomechanical Properties in Response to Pro-Inflammatory Stimuli

tissue type	inflammatory stimulus/ indicator	culture condition	dosage/duration	mechanical property	ECM change	ref
articular cartilage	IL-1α	explant culture	5 ng/mL 7 days	↓ dyn modulus ↓ equil modulus	↓ GAG ↓ collagen	68
articular cartilage	IL-1α	explant culture	5 ng/mL 7−21 days	↓ ultimate tensile strength ↓ tensile failure strain	↓ GAG ↓ Collagen	69
articular cartilage	IL-1α	explant culture	10 ng/mL 6 days		↓ GAG ↓ collagen	19
articular cartilage	IL-1α	explant culture	10 ng/mL 14 days	no change in dyn and equil modulus	no change in GAG or Collagen	92
articular cartilage	IL-1α	tissue engineered construct	10 ng/mL 7 days	↓ equilibrium modulus	↓ GAG	93
articular cartilage	IL-1α	tissue engineered construct	10 ng/mL 14 days	↓ dyn modulus ↓ equil modulus	↓ GAG ↓ collagen	92
articular cartilage	IL-1α	tissue engineered construct	10 ng/mL 14 days	↓ dyn modulus ↓ equil modulus	↓ GAG	91
articular cartilage	IL-1β	explant culture	10 ng/mL 6 days		↓ GAG ↓ collagen	19
articular cartilage	IL-1β	explant culture	10 ng/mL 28−48 days		↓ GAG	71
articular cartilage	IL-1β	explant culture	100 ng/mL 8 days	↓ equilibrium modulus	↓ GAG	70
articular cartilage	IL-1β	tissue engineered construct	10 ng/mL 7 days	↓ equilibrium modulus	↓ GAG	93
articular cartilage	IL-1β	tissue engineered construct	10 ng/mL 14 days	↓ dyn modulus ↓ equil modulus	↓ GAG	91
articular cartilage	collagenease	explant culture	0.1 wt % 120 min	↓ aggregate modulus ↑ permeability	↓ GAG	90
articular cartilage	↑ white blood cells in synovial fluid	clinical study		↓ dyn modulus ↓ aggregate modulus		81
IVD	TNF-α	3D organ culture	100 ng/mL 6 days	↑ dyn stiffness ↓ creep strain	↓ GAG ↑ collagen	86
IVD	TNF-α	3D organ culture	200 ng/mL 7 days		↓ GAG disrupted ECM organization	24
IVD	TNF-α & IL-1β	3D organ culture	100 ng/mL TNF 10 ng/mL IL-1 3−10 days		↓ GAG disrupted ECM organization	64
IVD	Papain	3D organ culture	150 U/mL 10 day	↓ compressive stiffness ↓rotational stiffness	↓ GAG	89
IVD	MMP3	3D organ culture	10 μg/mL 8 days		no correlation between disc height and GAG content	88
IVD	ADAMTS-4	3D organ culture	10 μg/mL 8 days		no correlation between disc height and GAG content	88
IVD	HTRA-1	3D organ culture	10 μg/mL 8 days		correlation between disc height and GAG content	88

Dyn stands for dynamic and equil stands for equilibrium.

inflammatory stimuli have been used in vitro and in situ studies, including TNF-α, IL-1α/β, and lipopolysaccharide (LPS). Therefore, this review will focus on the effects of these stimuli because they are the best studied pro-inflammatory cytokines (e.g., TNF-α and IL-1) in cartilaginous tissues. Understanding the current state of the field of inflammation and cell/tissue biomechanics may potentially identify future directions for novel and translational therapeutics with multiscale biomechanical considerations.

2. EFFECTS OF INFLAMMATION ON TISSUE BIOMECHANICS

2.1. Inflammation and Matrix Breakdown. Degenerative diseases are characterized by changes in biochemical composition and structure, which alter the tissue biological and biomechanical functions. The biological changes include loss of matrix protein such as proteoglycan and collagen, increase in macroscopic degenerative fibrillation, and altered water content.[45−51] Human degenerative IVDs exhibit higher levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and others compared to nondegenerative discs.[28,40,56,57] The upregulation of pro-inflammatory cytokines not only suppresses the synthesis of ECM proteins (e.g., collagen type II and aggrecan), but also stimulates the release of catabolic proteases (e.g., matrix metallopreoteinase MMPs, aggrecanases ADAMTS4/5).[27,58−60] The imbalanced anabolic-catabolic response to inflammation leads to further matrix breakdown and tissue deterioration, which can impair tissue mechanical

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function. Disc degeneration results in the NP changing from a gelatinous to a more fibrous structure, with the subsequent formation of clefts that eventually extend through the AF.[47] Both aging and degree of disc degeneration have been linked to increased levels of pro-inflammatory cytokines[40,61] which has itself been shown to up-regulate expression and activity of matrix degrading enzymes and promotes ECM degradation.[24,29,62−64]

Osteoarthritis (OA) is a disease characterized by degeneration of articular cartilage matrix, bone, synovium, and periarticular tissue.[65−67] Similar to degenerative disc diseases, OA involves a complex interplay of biochemical, mechanical, and genetic factors. In the joints of patients with OA, elevated levels of pro-inflammatory cytokines such as TNF-α and IL-1β are present and thought to be involved in the pathophysiology of OA.[32,33] These cytokines act on chondrocytes and other cell types in the joint to stimulate the production of matrix degrading enzymes and other inflammatory cytokines and mediators.[34−36] The increase in catabolic response in addition to decreased synthesis leads to net catabolic changes in matrix components. Studies investigating the effect of inflammatory stimulation on cartilage explants, with either IL-1α or IL-1β, have shown explants exhibit decreased proteoglycan content as well as increased presence of cleaved and denatured collagen.[19,68−71] Inhibitors of matrix degrading enzymes have also been shown to be able to prevent IL-1 induced changes to cartilage explant ECM indicating these inflammatory induced changes are dependent on the production of MMP's and increased ECM catabolism.[70] Following the upregulation of inflammatory mediators and catabolic proteases, there is an increase in collagen type II denaturation and type I procollagen synthesis in degenerated tissue.[45] Articular surface of cartilage becomes fibrillated with the presence of matrix cracks and vertical fissures.[66]

Degenerated cartilaginous tissues often have altered water content compared to healthy normal tissues. Aggrecan, one of the two main components of cartilaginous tissues, is a high-molecular-weight proteoglycan composed of glycosaminoglycan chains attached to a large core protein. Aggrecan exists as large aggregates that form from the interaction of hundreds of aggrecan molecules with hyaluronan.[47] Aggrecan aggregates are highly negatively charged molecules, which can absorb and retain water and provide compression-resisting properties of the NP and articular cartilage. Inflammatory factors disturb aggrecan homeostasis by decreasing its synthesis and increasing its catabolism by elevated levels of aggrecanases ADAMTS4/5, which could lead to a net catabolism of aggrecan. The decrease in aggrecan content with tissue degeneration results in differential changes in water content, depending on tissue types. For example, the decrease in aggrecan in the NP ECM directly leads to decrease in fixed charge density and decreased water content in degenerating IVD.[46] However, in articular cartilage, water content is increased with tissue degeneration. This is likely because water content in articular cartilage is regulated by a more complex interplay of collagen and aggrecan, with collagen network providing resistance to swelling tendency of aggrecan molecules. In degenerative cartilage, the collagen network is disrupted, resulting in an ECM with higher tendency to swell, and hence having higher water content.[50,51,72]

2.2. Inflammation and Tissue Mechanical Properties. Changes in ECM composition and structure of articular cartilage and of the IVD due to inflammation considerably change the mechanical properties of the whole tissues. Mechanical damage, aging, and trauma are believed to trigger inflammatory process in many tissues surrounding the joints and the IVD.[27,40,60,61,73−76] Mechanical loading, and specifically mechanical injury, regulates inflammatory signaling in articular cartilage (reviewed in refs 77−79). Effects of inflammation on cartilage mechanical properties have been studied through the context of OA and injury-induced trauma. Through different stages of OA, cartilage stiffness is changed dramatically depending on disease severity. During early stages of OA, cartilage in osteoarthritic joints has decreased shear modulus, tensile stiffness, and compressive strength compared to nonosteoarthritic tissue.[80−84] Additionally, differences in cartilage mechanical properties might be dependent on the degree of local inflammatory state, where increased white blood cell count in patients' synovial fluid is an indicator of reduced cartilage compressive stiffness.[81] As the disease progresses, there are increased surface fibrillation, further loss of aggrecan and collagen, and increased water content,[55] which result in further deterioration of mechanical integrity.[55,85]

Inflammation induces ECM catabolism through regulation of matrix degrading enzymes, such as MMPs and aggrecanases, which can have a deleterious effect on the mechanical properties of musculoskeletal tissues (see Table 1). In an organ culture model of intact bovine IVD, TNF-α treatment (100 ng/mL, 6 days) causes tissue stiffening as indicated by a 25% reduction in diurnal displacement and a 40−50% increase in dynamic stiffness from ∼2300 N/mm in unreated samples to 2800 N/mm in TNF-α-treated samples.[86] This response was likely dominated by the increased collagen staining rather than the reduced aggrecan content observed on histology. Similarly, AF tissue from degenerate IVD had a 2 fold higher compressive stiffness (∼1000 kPa) (i.e., aggregate modulus) compared to nondegenerate tissue (∼500 kPa),[87] which is likely related to condensed matrix associated with water loss during disc degeneration. While few other studies have directly observed the effects of inflammatory stimulation on IVD mechanical properties, treatment with MMPs or other matrix degrading enzymes such as papain has been shown to lead to overall tissue matrix disruption as well as decreased compressive and rotational stiffness of the bovine IVD organ culture.[88,89] Similarly, collagenase treatment (0.1 wt %, 120 min) of cartilage explants induced OA-like changes in tissue ECM including a 45% increase in tissue permeability (4.73 to 6.83 $m^4/(N\ S) \times 10^{-14}$) and a 50% decrease in aggregate modulus (0.13 to 0.06 MPa).[90] Unlike the IVD, there have been more studies in cartilage observing the direct effect of inflammatory stimulation on cartilage explant properties. Stimulation with IL-1α (5 ng/mL, 7 days) lead to an 80% decrease in compressive equilibrium (0.7 to 0.15 MPa) and a 70% decrease in dynamic moduli (7 to 2 MPa); similar effects are also seen with IL-1β treatement.[68,70] In another study, inflammatory stimulation with IL-1α (5 ng/mL, 21 days) was not found to affect cartilage explant tensile modulus but did reduce ultimate tensile strength by 30% (10 to 7.5 MPa) and tensile strain at failure by 33% (0.7 mm/mm to 0.4 mm/mm).[69] Similar effects have been observed in engineered cartilage constructs where inflammatory stimulation with IL-1α or IL-1β also shows decreased compressive equilibrium and dynamic modulus as well as decreased proteoglycan content.[91−93] Furthermore, studies comparing effect of IL-1α or IL-1β on dense cartilage explants vs less dense engineered cartilage show that engineered cartilage is more

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Table 2. Changes in Cellular Biomechanical Properties in Response to Inflammatory Stimuli

cell type	inflammatory stimulus	culture condition	dosage/duration	mechanical property	cytoskeleton change	reference
chondrocyte	IL-1β	2D	10 ng/mL 24 h	↑ stiffness	↑ F-actin	110
chondrocyte	TNF-α	2D	40 ng/mL 24 h	↑ stiffness	↑ F-actin	110
chondrocyte	IL-1α	3D	10 ng/mL 1 h		↑ F-actin altered F-actin distribution	109
chondrocyte	IL-1α	in situ	10 ng/mL 1 h		↑ F-actin altered F-actin distribution	109
nucleus pulposus	LPS	2D	1 μg/mL 24 h	↑ hydraulic permeability	altered F-actin distribution	107
nucleus pulposus	TNF-α	2D	10 ng/mL 24 h	↑ hydraulic permeability	altered F-actin distribution	107
annulus fibrosis	TNF-α	2D	10 ng/mL 24 h		↑ F-actin	108

susceptible to proteoglycan loss and decreased modulus following IL-1 stimulation.[92]

Changes in IVD mechanical properties seen during degeneration are likely affected by matrix fibrillation in addition to ECM degradation and altered hydrostatic pressure (Table 1). Although the role of inflammatory stimulation on hydrostatic pressurization has not been directly investigated, loss of hydrostatic pressure, swelling pressure, and aggregate modulus have all been shown to occur in degenerate human and mouse IVD[94,95] and can alter stress and load distribution in the IVD.[4] Interaction between tissue mechanical behavior and inflammatory signaling is bidirectional. Mechanical loading indeed has been shown to regulate inflammatory signaling in IVD tissues. Injurious loading such as static loading,[96] asymmetrical loading,[97] or superphysiological strain[73,98] induces increased levels of pro-inflammatory cytokines, which can further perpetuate degenerative changes seen due to injurious loading (reviewed in ref 54). Abnormal asymmetric loading, consisting of axial compression applied at a 15° angulation to IVD explants where the cartilaginous EP was removed, resulted in spatially dependent changes in inflammatory, ECM, and mechanical effects. Regions exposed to greater stress magnitude (convex side) exhibited increased pro-inflammatory gene expression of IL-1β, IL-6, MMP-1 and ADAMTS4 compared to the control symmetric loading conditions. Additionally, regions under convex loading had lower tissue compressive aggregate modulus compared to regions under concave loading.[97] Superphysiological cyclic loading has also been shown to increase pro-inflammatory cytokines in human NP and AF cells, as well as[98] in NP explant tissue to facilitate diffusion of inflammatory cytokines and inflammatory induced changes in NP tissue.[86]

Aging is a risk factor for OA. With advanced aging, loss of ECM hydration and associated PG content decrease the macroscopic compressive properties of cartilage.[50,99] At the nanoscale level, increased age results in production of altered aggrecan molecules, with shorter core protein length and shorter side chains.[100] Adult aggrecan was also found to be significantly weaker in compression than newborn aggrecan, even at the same total GAG density.[100] With age, changes to the collagen network occur, including increased fibril diameter, due to fibril bundling or loss of interfibrillar PGs, and increased collagen cross-linking.[101−103] At the macroscopic level, there is a decrease in the tensile-strength properties of cartilage with maturation.[104,105] However, at the nanoscale, aging results in a stiffer matrix when measured using nanoindentation.[106] These studies provide evidence of the effects of age on the structural and nanomechanical properties of cartilage ECM, with direct implications for alterations in cell−ECM interactions with age.

3. EFFECTS OF INFLAMMATION ON CELL BIOMECHANICS

3.1. Effects of Inflammation on Cellular Biology. Inflammatory stimuli can alter cytoskeletal components of cells in musculoskeletal tissues. The cytoskeleton of a cell consists of filamentous actin (F-actin), intermediate filaments such as vimentin, and tubulin microtubules, all of which play important roles in maintaining cell biological as well as mechanical functions. Cells from bovine NP of IVD when exposed to pro-inflammatory treatment in vitro exhibited altered F-actin cytoskeleton[107] (Table 2). Cells from AF region of IVD when treated with TNF-α in vitro showed an increased content of F-actin and a more diffusely connected α-tubulin network, but no change in vinculin.[108] Similarly, chondrocytes cultured in monolayer treated with TNF-α or IL-1 for 24 h increased the expression level of F-actin.[109,110] Alterations in cytoskeleton organization due to inflammation is also dependent on culture condition as well as the cellular microenvironment (2D vs 3D, Table 2). In normal NP cells and chondrocytes cultured in 3D, F-actin forms a bright solid ring around the periphery (cortex) of the cells. In situ chondrocytes have less organized F-actin compared to isolated cells and dispersed throughout the cells with focal areas of intense staining.[109] With inflammatory treatment, F-actin in isolated NP cells and chondrocytes becomes more punctate and the cortical localization of the filaments is no longer apparent.[107,109,111] F-actin organization in isolated cells treated with pro-inflammatory cytokines is similar in structure to F-actin when cells were treated with cytochalasin D, a reagent that disrupts actin polymerization.[111] IL-1α did not affect F-actin organization of chondrocytes in situ, but enhanced F-actin expression at the cell periphery.[109] When culture condition was changed to 2D, chondrocytes treated with TNF-α or IL-1β exhibited increased stress fiber formation.[110] These pro-inflammatory cytokines are also known to cause alteration in tubulin organization. AF cells when treated with TNF-α have a more diffusely connected microtubule network of α-tubulin.[108]

3.2. Effects of Inflammation on Cellular Mechanical Properties. Cytoskeletal changes induced by pro-inflammatory cytokines lead to significant changes in cellular biophysical

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properties (Table 2). Hydraulic permeability and size of NP cells treated with inflammatory factors, such as LPS or TNF-α increased significantly and remained elevated after 1 week post treatment in vitro.[107] A linear correlation was observed between hydraulic permeability and cell radius in untreated cells, but not in the inflammatory treated cells. The loss of correlation between cell size and hydraulic permeability suggests that regulation of biophysical properties of NP cells is disrupted irreversibly due to inflammatory stimulation in 2D in vitro culture.[107]

Alteration in cytoskeleton components also lead to marked changes in cellular biomechanical properties since these elements play major roles in regulating the mechanical properties of cells. F-actin is the main contributor to the cellular stiffness in many cell types while tubulin has a more minor contribution. Disruption of F-actin with cytochalasin D has been shown to result in reduction of chondrocyte stiffness.[110−112] The effect of cytochalasin D on chondrocyte stiffness is greater on healthy and normal chondrocytes than those isolated from osteoarthritic cartilage.[111] Chondrocytes treated with pro-inflammatory cytokines, such as TNF-α and IL-1β have 50% higher stiffness compared to untreated cells, which might be due to an increase in F-actin.[110]On the other hand, changes to other cytoskeletal elements such as vimentin under inflammatory conditions are unknown. In general, the contribution of vimentin to cellular physical properties remains debatable. In some studies, chondrocytes with disrupted vimentin using 4−5 mM acrylamide had a reduced stiffness compared to untreated cells,[112,113] while other studies showed that chondrocyte stiffness was unaffected by vimentin disruption using same treatment.[110,111] The differences in these studies could be due to different testing configuration and methods. In one study, cells were cultured and tested in 2D on tissue culture plastic,[110] whereas in other studies cells were cultured in alginate and tested in suspension[111] or in alginate.[113] The conflicting results of those studies suggest that microenvironment that cells are exposed to may change the phenotype of the cells, and hence, their cytoskeleton structure, cell morphology, and mechanical properties.

The physical interactions between cells and their microenvironment and how those interactions regulate the differentiation state of primary cells have been investigated in several studies using substrates with tunable mechanical stiffness. Studies demonstrate that the differentiation state of chondrocytes and immature NP cells of the IVD can be regulated by mechanical substrate stiffness.[114−119] Rat chondrocytes cultured in 2D on polyacrylamide gels with varying stiffness were found to express various degrees of collagen type I and collagen type II.[117] On the "stiff" substrate (40 kPa) with stiffness similar to the pericellular matrix surrounding chondrocytes, cells overexpressed Col-1 relative to Col-2, indicative of dedifferentiation.[117] When cultured on "soft" substrate (4 kPa) with stiffness on the same order of magnitude as the cell, chondrocytes produced higher level of SOX9, Col-2 and aggrecan.[117] NP cells from skeletally immature pigs cultured on "soft" laminin-containing basement membrane extract (BME, 0.3 kPa) produced more proteoglycans than NP cells cultured on rigid plastic surface coated with BME.[116] This protective effect of soft substrate on NP cell phenotype is dependent on N-cadherin expression.[120] Understanding how cells interact and respond to the in vitro mechanical cues via substrate stiffness may be helpful to further understand cell-matrix interaction in vivo. However, one limitation of such models is that the full repertoire of native cell−matrix interactions is not fully recapitulated, and thus interactions and compensatory mechanisms across varying cell−matrix interactions are difficult to assess.

Various studies have examined the effect of age on chondrocyte mechanical properties from model systems such as rabbit, bovine and from human cartilage.[112,121−123] Findings from these studies demonstrate a variety of trends that are dependent on species and/or method of testing. A recent study using a bovine model indicated that chondrocytes exhibit increased stiffness with maturation (between neonatal and adulthood) (from ∼0.5 kpa to 1 kPa), but not with further aging into late adulthood.[112] Steklov et al. have reported that the stiffness of chondrocytes obtained from older human individuals (>55 years old) was higher than chondrocytes obtained from younger human individuals (18−35 years old).[122] To the contrary, human OA chondrocytes obtained from older patients had an overall lower mechanical stiffness (0.037 N/m) than normal chondrocytes obtained from younger patients (0.096 N/m).[121] Thus, it is unclear if the modulation of the biomechanical properties is due to aging or disease. One interpretation could be that increases in elastic properties into adulthood are correlated with healthy aging. However, a peak in biomechanical properties may occur during maturation, followed by decline in cellular biomechanical properties in age-dependent degeneration.

Changes in cell mechanical properties could be interpreted as an adaptation to matrix stiffness changes, in order for cells to survive an altered microenvironment. Cytokines accelerate the degeneration of the extracellular matrix; consequently matrix stiffness is reduced and cell-matrix attachment may be altered. Under inflammatory conditions, cells from articular cartilage become stiffer and more resistant to compressive stress.[108,110] A possible explanation is that these cells experience and sense increased stress due to a reduction in surrounding ECM stiffness and compensate by becoming stiffer. One potential effect of the cytokine-induced cellular stiffening is that cells may become less sensitive to small perturbations/alterations in strain/stress. Through cytoskeletal reorganization, cells may alter their mechanical stiffness to adapt to the changes occurring in the ECM.

To address the adaptive hypothesis for multiscale biomechanical alterations in pro-inflammatory conditions, complex nonlinear multiscale computational biomechanical models are needed. Advances in computational biomechanics provide useful tools to study the complex interaction of cells and their microenvironment in many instances where it may be technically challenging (e.g., 3D real time strain analysis of cells under deformation in situ or in vivo) or impossible (e.g., quantification of stress states) to obtain experimental measurements. The finite element (FE) method provides a powerful approach to obtain the response of a complex system from individual contributions of elements. There is an extensive body of work on cellular mechanics of chondrocytes and NP cells using FE modeling.[124−131] Various multiscale FE models have been developed to simulate cell-matrix interaction under static[127,132] or transient/dynamic compressive loading,[133,134] to understand differences in cellular and tissue response to changes in matrix environment due to loading. Using FE modeling in combination with experimental testing, deformation behavior and mechanical properties of cells, as well as cell−matrix interaction could be further investigated, under healthy and degenerate conditions.[127,135,136] Development of such

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ACS Biomater. Sci. Eng. 2017, 3, 2644−2656

models for evaluation of inflammatory effects on multiscale biomechanical properties would further advance the state of understanding in the field.

3.3. Effects of Inflammation on Cellular Mechanotransduction. Alteration in cytoskeleton components and mechanical properties of cells due to inflammation may result in alteration in cell mechanobiology in response to stress or mechanical cues. One way that cells transduce mechanical signal is through deformation of the cytoskeleton.[137,138] Pro-inflammatory cytokines that could alter cytoskeleton components and cellular stiffness could also regulate the mechanoresponsiveness of cells to loading. Treatment with TNF-α or IL-1β resulted in a reduced contraction of chondrocytes in response to the contractile agonist histamine.[110] Higher stiffness and lower contraction responsiveness indicated that these chondrocytes are in a more contracted state even in the absence of mechanical stimulation. Exposure to IL-1β exacerbated the catabolic effect of pathophysiologically high tensile strain (18%) and prolonged (24 h) tensile strain on AF cells from rabbits.[139]

The change in cell mechanobiology and mechanosensitivity under pro-inflammatory conditions could be the result of altered mechanotransduction pathways. Cell surface channels, such as water channels and mechanosensitive ion channels are also regulated by pro-inflammatory stimulation. NP cells experience daily fluctuations in water content, with measurements showing 8% water loss upon loading, highlighting the importance of controlling water transport to withstand osmotic and volumetric changes in these cells and for disc function.[140] Bovine NP cells exposed to inflammatory stimuli in vitro such as LPS and TNF-α exhibited a decrease in aquaporin-1 expression.[107] Inflammatory induced reduction of aquaporin expression is consistent with findings in human IVD, where aquaporin-1 and aquaporin-5 expression decrease with increasing degeneration.[141] Transient receptor potential vallinoid-4 (TRPV4) is a calcium channel present in various cell types, including AF and NP cells of the IVD, articular chondrocytes, and cells from peri-articular tissues such as synovium, bone, and muscle.[142−147] Inflammation has an indirect effect on TRPV4 expression through modulation of tissue osmolarity. Treatment with TNF-α did not alter TRPV4 expression in isolated NP cells. However, NP and AF cells in whole IVD cultured in media containing TNF-α showed an increase in TRPV4 expression.[143] Taken together, these results suggest that the upregulation of TRPV4 in disc cells is likely due to reduction in tissue osmolarity following proteoglycan degradation induced by exposure to pro-inflammatory cytokines such as TNF-α. Degenerated human IVD and cells in synovium of early OA also showed an increased expression of TRPV4 compared to healthy tissue.[143,146] TRPV4 is believed to play a central role in the cellular signal transduction in response to mechanical or osmotic stimulations. Inhibition of TRPV4 during dynamic loading prevented up-regulation of pro-anabolic and anticatabolic genes and attenuated the enhancement of matrix accumulation and mechanical properties of chondrocyte-embedded agarose constructs.[144] Another family of ion channels implicated in tissue/cell injury is the PIEZO family. PIEZOs are cation-permeable channels that can be activated directly by mechanical signals. PIEZO1 and PIEZO2 are abundantly expressed in normal articular chondrocytes from mice, pigs, and humans as well as in other tissues.[148] High strain ($\geq$50% deformation) applied to articular chondrocytes causes increased intracellular Ca^{2+} influx through PIEZO1 and PIEZO2, which is dependent on actin polymerization.[148] Although it has been shown that PIEZO channels involved directly to the mechano-transduction of primary chondrocytes in response to high mechanical strain, how pro-inflammatory cytokines affect PIEZO expression and function remains to be fully investigated.

Integrins are heterodimeric transmembrane glycoproteins, consisting of α and β subunits, and are major mechanoreceptors in various tissues within the body.[149] Similar types of integrins have been identified in articular chondrocytes and cells from NP and AF regions of the IVD.[150−152] Integrin-mediated mechano-transduction involves recognition of the mechanical stimulus by integrins and activation of integrin-mediated signaling pathways leading to biochemical changes. Studies have shown that integrin expression was increased when cells were cultured in inflammatory conditions.[153,154] Evidence also exists to support that articular chondrocytes, NP and AF cells from healthy tissues respond to mechanical cues through integrin-mediated pathway, whereas mechanotransduction in similar cell types from degenerate tissues involve different signaling pathways that is not dependent on integrins.[155−158] The use of different mechano-transduction pathways by cells isolated from tissues with different health status (heathy vs degenerative) may explain different responses of these cells to similar mechanical stimulation. For example, dynamic compression induced proteoglycan synthesis in normal chondrocytes, whereas similar loading induced a catabolic response in chondrocytes from OA cartilage indicated by upregulation of IL-1β and IL-6.[15,158−160] Cyclic tensile strain at physiological frequency (1.0 Hz) induced an anticatabolic response in AF cells isolated from nondegenerated human IVD as indicated by decreased expression of matrix degrading enzymes such as MMP-3 and ADAMTS-4 with no change in expression of matrix genes such as aggrecan and collagen II. Similar loading condition, however, induced a catabolic response in AF cells from degenerated human discs, as indicated by a down-regulation of aggrecan.[161,162] Similar differential effects of mechanical stimuli were also observed in AF cells from rabbits.[163] Moreover, human NP cells derived from degenerate IVDs exhibited a lack of response to hydrostatic pressure, in contrast to the anabolic response observed in cells derived from nondegenerate IVDs.[164] Taken together, these studies suggest that degenerative changes in surrounding microenvironment due to inflammation or disease not only change how the cells respond to mechanical signals (e.g., through different pathways), but also decrease their mechano-sensitivity.

3.4. Disease Modifying Drugs. Several anti-inflammatory, anticatabolic, and pro-anabolic drugs to treat OA and disc degeneration have been recognized as potentially useful therapies to reverse or prevent matrix breakdown and progressive tissue degeneration.[21,165−167] Anti TNF-α therapy using etanecerpt, infliximab, and others has been shown to improve pain and behavioral responses in human clinical trials on patients with radicular pain due to lumbar spinal stenosis and patients with sciatica [reviewed in ref 21]. Anticatabolic glucocorticoids, pro-inflammatory cytokine inhibitors (IL1-ra, anti-TNF-α), MMP inhibitors, and pro-anabolic growth factors (IGF-1, FGF-18, and BMP-7) are disease modifying drugs under investigation for efficacy using cartilage explants.[168−171] The use of anti-inflammatory agent Flavopiridol,[172,173] an inhibitor of the transcription factor cyclin-dependent kinase 9 (CDK9) has shown efficacy in protecting cartilage from

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ACS Biomater. Sci. Eng. 2017, 3, 2644−2656

inflammatory induced decrease in PG content and loss of compressive stiffness in vitro.[172] Alternatively, the use of the chemical cross-linking agent Genepin has been shown to protect the compressive stiffness of engineered cartilaginous constructs against pro-inflammatory cytokines.[174] Due to the avascular nature of articular cartilage and the IVD, systemic administration of these drugs could offer limited effects as a majority of the active molecules may degrade before sufficient diffusion or accumulation into the targeted tissues.

Several approaches using micro- and nanocarriers have been developed for sustained delivery of small drug molecules locally and directly to the target tissue.[175−182] Chitosan-based thermosensitive hydrogels have been investigated as drug depots of anti TNF-α therapeutics, which would allow local and sustained release and may increase the efficacy of anti TNF-α treatment.[183] Upon intra-articular injection of siRNAs (inhibiting TNF-α) encapsulated in poly(dl-lactide-*co*-glycolide) (PLGA) microspheres, siRNAs was slowly released and effectively inhibited the expression of TNF-α in murine arthritic joints.[180] Similarly, intra-articular injection of IL1-ra encapsulated in PLGA microspheres inhibited joint inflammation in an anterior cruciate ligament transection (ACLT) rat model as indicated by a reduction in lymphocyte proliferation and cartilage degradation. Cartilage and synovial histopathology scores were also reduced. Serum levels of IL1-ra were also significantly lower with injection of PLGA/IL1-ra compared to free IL1-ra.[178] Encapsulating dexamethasone, an anti-inflammatory drug, in superparamagnetic iron oxide nanoparticles (SPIONs) demonstrated an increased joint retention and similar anti-inflammatory effect to free dexamethasone in a mouse model.[182] When translating the findings of these studies to clinical application, challenges with diffusion and dosage due to animal sizes and clearancepresent potential limitations of these small animal models. Taking advantage of the negative charge density of articular cartilage, Avidin and other polypeptides are also being investigated as positively charged drug carriers into cartilage, where the negatively charged cartilage matrix facilitates Avidin's retention.[175,176] Charge-based intracartilage delivery of a single dose of dexamethasone using Avidin nanocarriers was found to suppress IL-1α induced catabolism long-term.[176] These local delivery systems confirm that rapid drug penetration, sustained release, and prolonged joint retention within the target tissue can be efficacious at protecting tissue biomechanics and biochemistry from pro-inflammatory insults.

5. CONCLUSION

In summary, inflammation following mechanical injury or disease causes up-regulation of pro-inflammatory cytokines in cartilaginous tissue, which not only suppresses the synthesis of ECM proteins, but also stimulates the release of catabolic proteases. At the cellular and subcellular levels, inflammation causes disruption of cytoskeleton structure, altered cellular biomechanical and biophysical properties, and changes in the expression of water and ion channels. These changes could potentially lead to impaired cellular functions in maintaining tissue homeostasis. The imbalanced anabolic-catabolic response to inflammation leads to matrix breakdown and tissue deterioration, which impair tissue mechanical function.

A growing body of research has focused on the effects of inflammation on various cartilaginous cell and tissue properties. However, the relationship between cellular property alterations and macroscopic tissue level alterations has not been delineated. As knowledge of the effect of inflammation on mechanical properties of cartilaginous tissue as well as on the biophysical properties and phenotypes of cells within these tissues continues to grow, we will be able to form a more complete picture of how inflammation-induced changes compare to changes observed within diseased states. Understanding disease mechanism in terms of cell and tissue biomechanics will enrich experimental therapeutic approaches to mitigate and potentially reverse disease progression.

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Notes

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