Mechanobiology of Hyaluronan:
Connecting Biomechanics and Bioactivity in Musculoskeletal Tissues

[1,2,†]Deva D. Chan
0000-0003-1508-1045

[3,4]Farshid Guilak
0000-0001-7380-0330

[5]Robert Sah
0000-0002-5493-9502

[6]Sarah Calve
0000-0002-7887-6307

[1]Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana, USA
[2]School of Mechanical Engineering, Purdue University, West Lafayette, Indiana, USA
[3]Washington University in St. Louis, St. Louis, Missouri, USA
[4]Shriners Hospitals for Children – St. Louis, St. Louis, Missouri, USA
[5]Shu Chien-Gene Lay Department of Bioengineering, University of California at San Diego, San Diego, California, USA
[6]Paul M. Rady Department of Mechanical Engineering, University of Colorado Boulder, Boulder, Colorado, USA

[†]Corresponding Author: chand@purdue.edu, 765-496-1768 (o), 765-496-1459 (f)

Abstract: Hyaluronan (HA) plays well-recognized mechanical and biological roles in articular cartilage and synovial fluid where it contributes to tissue structure and lubrication. An understanding of how HA contributes to the structure of other musculoskeletal tissues, including muscle, bone, tendon and intervertebral discs is growing. In addition, the use of HA-based therapies to restore damaged tissue is becoming more prevalent. Nevertheless, the relationship between biomechanical stimuli and HA synthesis, degradation, and signaling in musculoskeletal tissues remains understudied, limiting the utility of HA in regenerative medicine. In this review we discuss the various roles and significance of endogenous HA in musculoskeletal tissues. We use what is known and unknown to motivate new lines of inquiry into HA biology within musculoskeletal tissues and in the mechanobiology governing HA metabolism, by suggesting questions that remain regarding the relationship and interaction between biological and mechanical roles of HA in musculoskeletal health and disease.

Keywords: hyaluronan, biomechanics, extracellular matrix, mechanobiology, musculoskeletal, mechanotransduction

1 Introduction

Hyaluronan (HA) is a ubiquitous extracellular matrix (ECM) molecule in many solid tissues of the body, as well as certain biofluids. The simplicity of a repeating disaccharide chain belies HA's myriad functions throughout the body; HA plays major roles in development, homeostasis, and the response to injury (1). Many of these roles arise from the differential synthesis of HA, both in terms of its molecular weight and localization and interactions after synthesis in the pericellular space, as well as its breakdown, turnover, and interactions with other matrix components. In addition, many factors can regulate HA metabolism, such as the availability of precursors, cytokine signaling, and physical cues. The role of HA in various tissues, including blood vessels, lung, and kidney, and health states, including inflammation and cancer, have been studied; however, despite the long-appreciated role of HA in cartilage and synovial fluid, the mechanical and bioactive roles of HA in other musculoskeletal tissues is understudied. This review will provide an overview of our current knowledge of HA in a number of musculoskeletal tissues, with particular emphasis on those associated with the synovial joint and of the intersection of mechanical cues and responses (i.e., mechanobiology) with HA metabolism. We propose that improved understanding of the interrelationship between HA biology and mechanobiology is vital to understanding the physiology and pathophysiology of musculoskeletal tissues. Identifying the tissue-specific effects and role of HA is critical for the success of biomedical engineering applications of HA-based tissue repairs, replacements, or drug delivery strategies, which are becoming increasingly prevalent for the treatment of a spectrum of joint diseases (2).

1.1 Hyaluronan in the ECM

HA is a nonsulfated glycosaminoglycan (GAG) consisting of repeating disaccharides that is found in nearly all vertebrate solid tissues and fluids, including the human musculoskeletal system (**Error! Reference source not found.**). The linear structure of HA coils randomly and its negative charge enables retention of high fluid volume, supporting osmotic pressure. HA is an integral part of the ECM, where it can be found directly surrounding cells in a localized pericellular matrix (PCM). It is also found within the interstitial matrix, between individual collagen fibrils (3) and acts as a space-filling amorphous hydrogel by complexing with proteoglycans (PGs) (4). Lecticans (*e.g.* aggrecan) are a family of PGs that are characterized by multiple, long GAG chains extending from a protein core to bind between a globular domain on the N-terminal of the protein core to HA, which is stabilized by Link protein. HA also interacts with and aggregates other matrix molecules, including bikunin, inter-alpha-trypsin-inhibitor proteins (IαI), and TSG-6 (encoded by gene TNFAIP6) to further stabilize PCM and ECM (5), as reviewed by others (see Related Resources). The density of HA and the composition of the binding partners is an important factor that varies among different tissues and changes as a function of development and disease, suggesting that modulation of HA can have significant effects on tissue properties and function under physiologic or pathologic conditions.

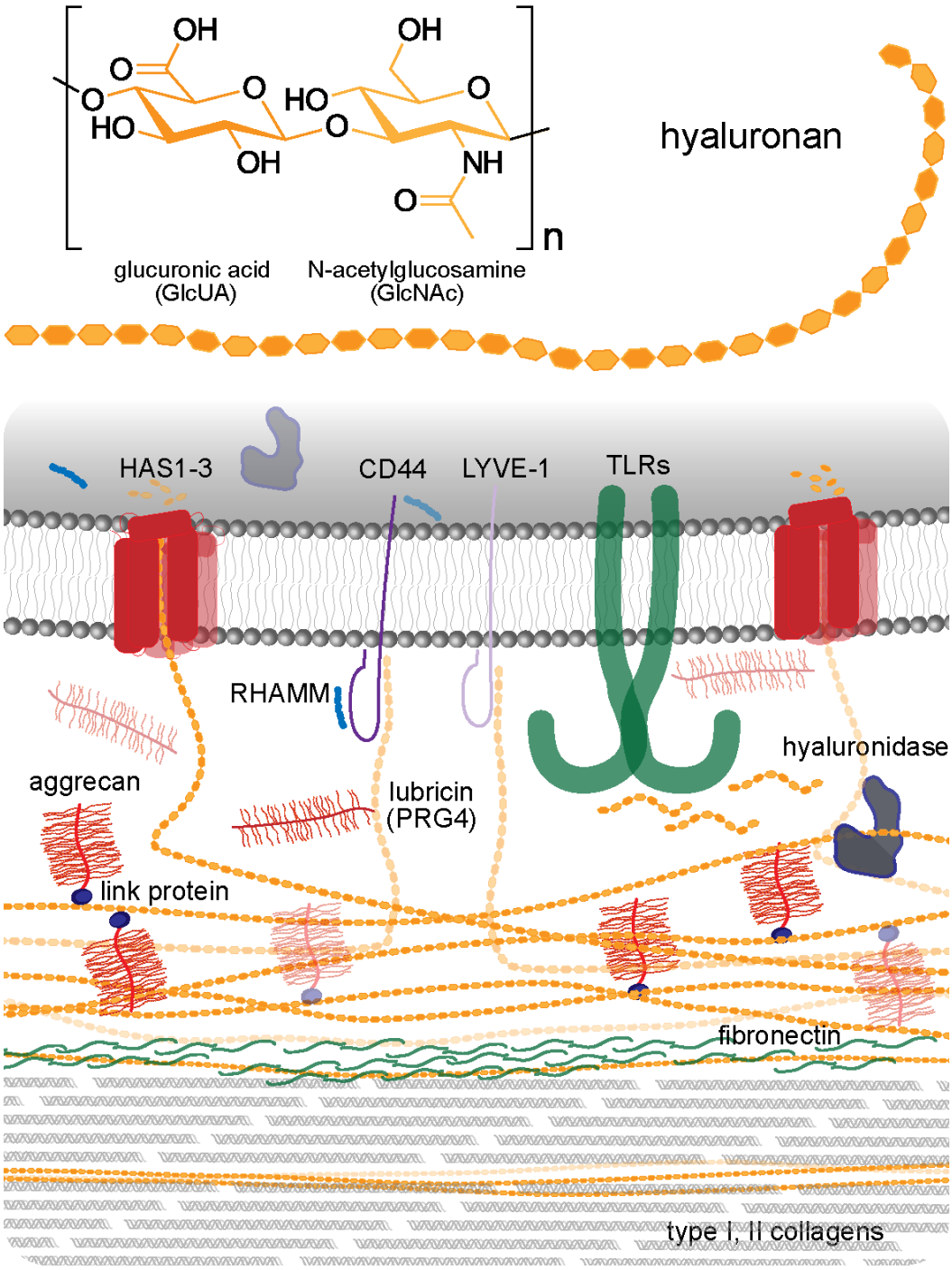

Figure 1. Hyaluronan (HA) is a high molecular weight glycosaminoglycan, made up of repeating dimers of glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc; top). Intracellular GlcUA and GlcNAc are assembled into long, linear chains by hyaluronidase synthases 1-3 that reside on the plasma membrane and HA is directly extruded into the extracellular space. Various receptors and ECM can bind HA and those discussed in the text are highlighted in the schematic (bottom).

1.2 Hyaluronan Synthesis and Breakdown

Turnover and removal of HA affects biomechanics by altering the composition of tissues and the interaction of HA with other ECM components. HA is synthesized at the cell membrane by hyaluronan synthases (HASs) as a long chain GAG made up of alternating N-acetyl-β-D-glucosamine (GlcNAc) and β-D-glucuronic acid (GlcUA) disaccharide units. There are three different HASs (HAS1-3), which are differentially regulated at multiple levels (transcription, translation, post-translation) (6). The isoforms produce HA of differing molecular weight and localization on the cell surface after synthesis (7; 8), and are differentially expressed among cell types (9). HA produced by HAS1 or HAS2 ranges from 2×10^5 to 2×10^6 Da, while HAS3 produces HA chains between 1×10^5 and 1×10^6 Da (10). Breakdown of HA occurs by free radicals (11-13) and catabolic enzyme activity (14). Degradative enzymes that act on HA include hyaluronidases 1-4 (HYAL1-4), cell migration-inducing hyaluronidases (CEMIP, also known as HYBID or KIAA1199, and CEMIP2, also known as TMEM2), and PH20 (15).

1.3 Hyaluronan Receptors and Interactions

The primary receptors for HA associated to musculoskeletal tissues are CD44 (16) and RHAMM (17), although other HA receptors are found elsewhere in the body, including LYVE-1, stabilin-2/HARE, and layilin. CD44 (cluster of differentiation 44) is a transmembrane glycoprotein and the major HA receptor in most cell types (18-21), interacting with PCM and ECM macromolecules and mediating cell-matrix crosstalk (22). Aside from anchoring cells to their matrix via HA, CD44 also functions to regulate cell migration, proliferation, and a host of other cell functions that are well reviewed elsewhere (see Related Resources). RHAMM also binds to HA near the cell surface and is associated with not just cell motility but also tissue responses to injury. RHAMM has also been shown to bind to both extracellular and intracellular macromolecules, suggesting a multitude of functions in cell signaling and cell-matrix interactions (23). HA interactions with both its receptors at the cell membrane and molecules in its pericellular and extracellular environments thus affect not only the tissue mechanics but also cell-matrix interactions that enable mechanotransduction (24).

2 Hyaluronan in Musculoskeletal Tissues

Although HA is present in all musculoskeletal tissues, its synthesis, turnover, and other aspects of its biology differ by tissue type, development stage, and health status. We will focus our discussion mainly on the tissues of the synovial joint, starting with HA synthesis, breakdown, and interactions within synovial fluid and the synovium and moving to articular cartilage, bone, tendon and ligament, and skeletal muscle, before briefly summarizing what is known about HA in other musculoskeletal connective tissues (Figure 2). This section covers the tissue-specific role of HA in musculoskeletal biomechanics, whereas Section 3 will focus on HA and mechanobiology.

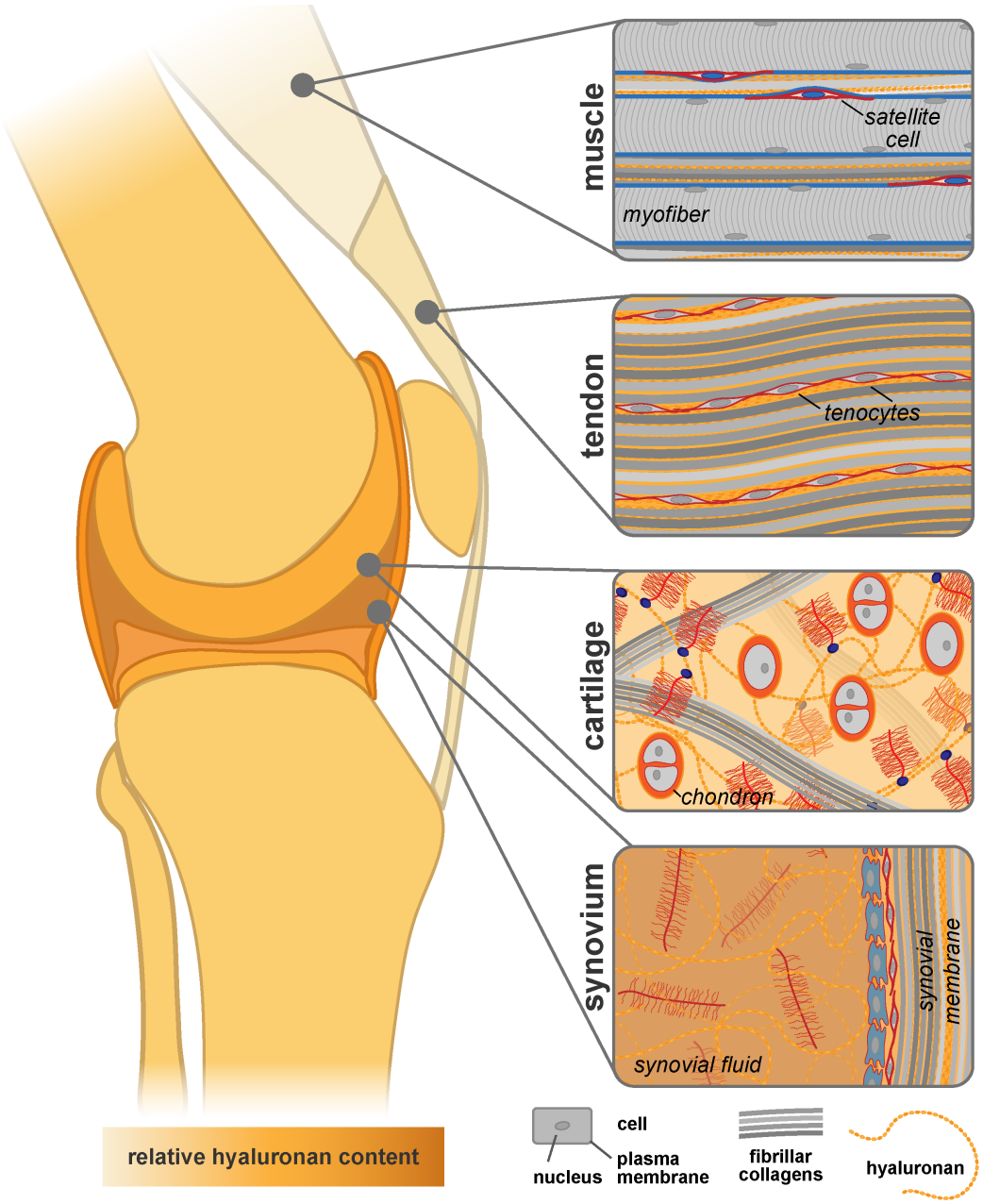

Figure 2. The distribution and concentration of HA varies across musculoskeletal tissues. The relative distribution of HA, fibrillar collagens and cells are shown for 4 representative tissues.

2.1 Synovium and Synovial Fluid

Intraarticular synovial fluid is produced by the synovium, also known as the synovial membrane, and other tissues lining the joint. The synovial membrane consists of macrophage-like (type A) and fibroblast-like (type B) synovial cells, or synoviocytes within a collagenous ECM (25-27). Healthy synovial fluid is associated with high content (2-3 mg/mL) and molecular weight (2.5 MDa or greater) of HA (28-30), and overall concentration at all molecular weight ranges decreases with age, even in the absence of known joint disease (30). This viscous solution works to reduce friction between the articulating surfaces of the joint and is a depot for nutrients and signaling molecules (31). HA also helps to resist interstitial fluid drainage during joint pressurization (32), a mechanism termed outflow buffering that is attributed to the osmotic pressure generated by high molecular weight HA (33).

The lubricating ability of synovial fluid is due largely to HA and PRG4 (or lubricin), secreted by both chondrocytes and synoviocytes (34; 35). HA and PRG4 form a complex that contributes synergistically to synovial fluid's role in facilitating cartilage lubrication (31; 36-39). When PRG4 interacts with high HA concentrations, viscosity is reduced; however, PRG4 increases synovial fluid viscosity at low HA concentrations (40). HA also complexes with surface-active phospholipids in synovial fluid to contribute to boundary lubrication at the cartilage surface (31; 41).

In synovial tissues, HAS1 mRNA was reported to be the most highly expressed isoform, with HAS activity and utilization in uncultured synovial tissues similar to that of cultured cells (9). Notably, increased expression of the HA synthases did not directly correlate to increased HA production, indicating that control of HAS gene expression and mRNA levels is only one aspect of controlling HA biosynthesis (9). Inflammatory cytokines may also modulate the synthesis of HA in the synovium, with synoviocytes responding robustly to interleukin 1 beta (IL-1β) through increased HAS gene expression, both in the presence of IL-1β only and in synergy with transforming growth factor beta (TGFβ) signaling (9). Separately, cultured rabbit synovial membrane cells increased gene expression of HAS2 and HAS3 when stimulated by IL-1β and tumor necrosis factor alpha (TNFα), with interferon gamma (IFNγ) also increasing HAS3 transcription (42). An increase in the precursor GlcNAc also enhanced HA production in explants from human osteoarthritic (OA) synovium (43). In addition to the production of HA in synovial fluid, synovial fibroblasts may generate HA-coated extracellular vesicles, which have been documented in human synovial fluid (44), potentially derived from the long HAS+ cellular protrusions.

The half-life of HA within synovial joints has been estimated to be from about 10 to 24 hours, depending on the measurement technique and the health status of the joint (45; 46). Degradation of HA by free radicals (11; 12) and catabolic enzyme activity contribute to this high turnover rate. HYAL1 and HYAL2 genes are expressed in synovial cells, although activity levels *in vitro* are low in the absence of load (47).

Colocalization of HA and CD44 is a hallmark of the synovial lining (16; 21). CD44 expression decreased in joints affected by rheumatoid arthritis (RA), and RA synoviocytes showed a reduction of CD44+ cytoplasmic extensions when cultured (18). RHAMM expression, on the other hand, was greater in a murine model of inflammatory arthritis than control and higher in RA compared to OA fibroblast-like synoviocytes (48). Both CD44 and RHAMM expression increased with human OA in knee synovial tissue (49).

The content and molecular weight distribution of HA in synovial fluid are altered with aging, injury, and diseases such as OA and RA (30; 50-52). In general, the distribution of HA molecular weight shifted downward with cartilage injury (51) and degeneration (30) and osteoarthritis progression (50; 53). In addition to already being highly expressed in healthy synoviocytes (9), HAS1 was also upregulated with OA synovitis (54). Synovial fluid hyaluronidase activity increased, with both rheumatoid arthritis and OA, and correlated with markers of synovitis (55). Hyal2 expression increased in OA, shifting the molecular weight distribution lower (56). CEMIP expression increased with OA (57) and was associated with inflammation, fibrosis, and synovial hyperplasia via TGFβ pathway (58). Altered content and reduced molecular weight of HA in synovial fluid result from an imbalance between synthesis and degradation, leading to higher friction coefficients within the joint (59).

2.2 Articular Cartilage

Articular cartilage is the thin layer of load-bearing material that lines the bony ends of all diarthrodial joints. The primary functions of this tissue are to support and distribute forces generated during joint loading and to provide a low-friction surface to prevent wear of the joint with physiologic movement and loading. HA is concentrated around the immediate pericellular space of articular chondrocytes, a specialized PCM that makes up, along with the cell itself, a functional unit known as a chondron (24; 60). HA also exists within the ECM, beyond the PCM, where its primary structural function is to promote aggregation of proteoglycans. HA has long been recognized to contribute to the mechanical behavior of articular cartilage. Within the cartilage ECM, aggrecan molecules are bound to a single long chain of HA to form large proteoglycan aggregates that also immobilize and restrain it within the fibrillar type II collagen network. Furthermore, HA generally serves a scaffold-like role that integrates many other ECM molecules (61). Because of the large net negative charge of aggregated PGs, cartilage is highly hydrophilic, with a tendency to imbibe fluid and "swell," to maintain a physicochemical and mechanical equilibrium (62; 63). This property significantly contributes to the mechanical function of articular cartilage by generating a large swelling pressure that facilitates load support and tissue recovery from deformation (64).

In addition to aggregating PGs in cartilage, high molecular weight HA has been shown to be protective of cartilage through the inhibition of the expression and/or activity of ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs). HA-driven inhibition of ADAMTS9 mRNA expression was associated with blockage of aggrecan cleavage in OA rats (65). Others have found that HA formed a complex with ADAMTS5, acting to sequester this aggrecanase within the PCM (66). Similar in effect to direct binding/sequestration of ADAMTS in cartilage/chondrocytes, the presence of high molecular weight HA was involved in the suppression of ADAMTS4 expression by synoviocytes (67).

In healthy cartilage, HA is primarily produced by HAS2 and is required for PG aggregation and retention (68). HAS3 has also been detected in chondrocytes (69), though at significantly lower transcript levels (70; 71). The inhibition of HAS2 using antisense mRNA in human articular chondrocytes, in turn, inhibited PG retention and matrix assembly (70), further evidencing HAS2's role as the primary synthase in healthy cartilage. Changes to HAS1-3 relative gene expression levels were also a hallmark of articular chondrocyte de- and redifferentiation when primary cells were grown in culture (72). Dedifferentiated primary chondrocytes shared a common pattern of HAS mRNA expression, which were altered during passaging and expansion, but returned to levels equivalent to passage-0 and tissue chondrocytes

with pellet culture (73). However, HAS enzymatic activity levels in uncultured cartilage were like those in cultured cells (9). Unsurprisingly, these shifts in HAS expression also correlated with changes in molecular weight of HA synthesized by cells in culture (73). Together, these indicated that HAS expression is tied to chondrocyte phenotype and tissue health.

Hyal1 and Hyal2 are the constitutively active hyaluronidases in healthy cartilage with the latter expressed at higher levels by chondrocytes (74). Both enzymes are expressed in early cartilage matrix formation and hypertrophic cartilage in costal cartilage (75), indicating that hyaluronidase expression and activity are critical to chondrocyte differentiation. CEMIP expression has also been detected in primary chondrocytes (76). Catabolism of HA is also key to cartilage homeostasis, as conditional knockout of Hyal2 in cartilage stimulated OA progression in mice (77). Some studies suggest that intracellular localization of Hyal2 indicates that HA degradation in chondrocytes is primarily dependent on receptor-mediated endocytosis and delivery of HA to low-pH organelles containing hyaluronidases (78), albeit not without controversy (74; 79). A later study showed that CD44 and HYAL2 were also bound at surface of chondrocytes, where HYAL2 was released when CD44 is shed (80). Interestingly, HA fragments also activated the production of nitric oxide, via nitric oxide synthase, by chondrocytes (81), potentiating HA degradation via reactive oxygen species.

Beyond aggregation of PGs in the cartilage matrix, HA interaction with its receptors and other ECM also modulates the mechanics of cartilage. HA receptors facilitated the directed assembly of chondrocyte PCM (82) and pericellular HA of chondrocytes (83) (reviewed in (61)), where the introduction of GlcNAc and GlcUA to competitively bind HA receptors resulted in the inhibition of PCM aggregation of PGs (83). CD44-HA interactions also play a major role in a variety of signaling pathways related to chondrocyte proliferation, matrix synthesis, and remodeling (84)

Alterations to cartilage homeostasis are often associated with disease. Lower HA MW distribution is associated with a risk of osteoarthritis (OA) progression (50). Intriguingly, it is the overall turnover, and not simply HA synthesis, that is associated with disease. The residence time of [^{3}H]HA in rabbit stifles increased in half-life with OA, indicating a reduction in HA turnover. Increased expression of CEMIP and TMEM2 in chondrocytes is associated with OA inflammation and fibrosis (85-88). Similarly, Hyal2 conditional knockout mice demonstrated increased OA progression compared to wildtype (77).

2.3 Bone

Bone consists of mineral, organic matrix, and water phases, with the mineral phase consisting of primarily hydroxyapatite and the matrix phase comprised of type I collagen, fibronectin, and other molecules (89). Bone is typically classified as either cortical (compact) or trabecular (cancellous or spongy) at the macroscale; however, the mineral-matrix composite is common between cortical and trabecular bone at the cell scale. Homeostatic bone remodeling occurs through a process of osteoclast-driven resorption of bone and osteoblast deposition of new ECM, enabling bone to adapt to altered mechanical loading. In contrast to synovial fluid and cartilage, the contribution of HA to overall mechanical behavior of bone is less clear. Nevertheless, there is growing evidence that HA plays an important role in bone biology (90). HA is implicated in bone development, particularly during endochondral ossification (76; 91; 92), a premise strongly supported by the role that HA plays in cartilage. All three HAS isoforms are involved in endochondral ossification (93), suggesting that changes to HA synthesis could impact bone formation. Indeed, genetic knockout of Has1 and Has3 in a mouse model resulted in different

matrix and mineral content and mechanical behavior (94). In addition, osteogenesis imperfecta is a collagen-related bone disorder that is marked by altered hyaluronan content (95), manifesting in a reduced mineral-to-matrix ratio (96) which impacted tissue mechanics. (97) Downregulation of *HAS3* and reduction in HA production compared to non-mineralizing cells has been shown in human mineralizing osteoblasts (97), suggesting a link between mineralization and HA production by Has3 in mice. Exogenous high molecular weight HA also stimulated osteoblast activity, increasing mineralization (98). HA synthesis increased in a dose-dependent manner in response to parathyroid hormone (PTH) and was also correlated with PTH-induced bone resorption (99; 100), although this effect may have been via metabolic pathways rather than through demineralization processes (101). Together, these reports supported the hypothesis that HA modulates osteoclast-mediated bone resorption (102). In contrast, even less is known about the role of HA catabolic enzymes in bone. CEMIP enhanced recruitment and migration of osteoblastic stem cells (103) but also inhibited osteoblast differentiation (104). A deficiency in Hyal1 resulted in decreased bone mineral density, femoral length, and altered osteoblast and osteoclast activity (105).

HA receptor interactions may also alter bone structure and mechanics. Binding of HA to CD44 may modulate osteocyte migration and cell-cell communication (106). HA may inhibit osteoclastogenesis of precursor cells via TLR-4 binding (107). Some studies demonstrated that HA stimulates expression of receptor activator of nuclear factor κB ligand (RANKL), an osteoclast differentiation factor, via CD44 (108; 109), while another showed that enzymatic degradation of HA in and around bone marrow stromal cells promoted osteoclast formation (110). While siRNA knockdown of HAS2 enhanced dexamethasone and 1,25-Dihydroxyvitamin D_3 induced RANKL expression, RANKL expression was down-regulated by TGF-β1 induction of HA synthesis (110).

2.4 Tendon and Ligament

Tendons and ligaments are predominantly made up of aligned, type I collagen fibers and are relatively acellular compared to muscle and bone. Linear arrays of tenocytes reside in between collagen fibers and are responsible for maintaining and remodeling the surrounding ECM. In areas of tensile loading, HA expression is low, whereas regions that undergo compression have increased HA and PGs to resist transverse compression (111). Removal of compression results in a decrease in HA in fibrocartilages, whereas removal of tension increases HA in tensile regions (112).

HA is the predominant GAG in fetal tendons, but concentration decreased rapidly during maturation (113; 114), and was hypothesized to minimize collagen fibril diameter during early tendon (and other connective tissue) growth (115). In addition, the rapid decrease in HA/increase in fibril diameter correlated with the onset of muscle activity in multiple animals (116), suggesting an influence of mechanical loading on fibrillogenesis. *In vitro* studies support these hypotheses; the addition of HA to cultured rabbit Achilles tenocytes downregulated collagens at the gene level as well as lysyl oxidase (117). Nevertheless, the effect on proliferation remains unclear; *in vitro* exogenous application of HA to tendon fragments (118) and isolated cells reduced cell proliferation (119; 120). In contrast, it was reported that HA increased tendon cell viability and proliferation *in vitro* (121).

Knockout of Has1 and Has3 did not substantially affect the development or material properties of the murine Achilles tendon; however, the retrocalcaneal bursa (which separates and protects the Achilles from the calcaneus) was absent when Has1 was knocked out (122).

Notably, Has3 mRNA was not detected in this study (122), but was upregulated in a rabbit model of injury (123).

Various forms of injury lead to an increase in HA, including acute fatigue (124), tendon rupture (125), or experimental repair (126). Has2 and Has3 were significantly upregulated for the first two weeks in a rabbit model of flexor tendon injury, which are hypothesized to potentially help with adhesion formation, or as a target to prevent adhesion formation (123). CD44 is present in tendon cells and is upregulated during healing of adult tendons (127). Notably, knockout of CD44 in mice appeared to improve the mechanical properties of injured patellar tendons, leading the authors to conclude that since HA is degraded primarily through CD44, removal of CD44 may help increase HA in the injured environment and reduce scar formation (128). CD44 and apoptosis were upregulated in biceps tendinopathy; when CD44 was antagonized via anti-CD44 monoclonal antibody OX-50 in a rat model of inflammation (induced by injection of collagenase), the number of apoptotic cells significantly increased along with other inflammatory mediators, whereas the expression of collagens I and III were decreased. The authors conclude that CD44 expression and activation attenuates the inflammatory response (129). Hyaluronidases also likely play a role in clearing out excess HA, as Hyal2 was significantly upregulated in equine tendonitis (130); however, similar to bone, little is known about hyaluronidases in tendon.

2.5 Skeletal Muscle

Myofibers, the cells responsible for muscle contractility, are multinuclear, striated and densely packed with contractile machinery. Satellite cells, the mononuclear adult muscle stem cell, lie quiescent on the myofiber surface. Surrounding individual myofibers and the resident satellite cells is a laminin-rich basement membrane, which is linked to fibrillar collagens and elastic fibers embedded in an amorphous matrix of HA and PGs (131). Even though it appears that HA lies outside of the basement membrane, in homeostatic muscle, HAS1-3, Hyal1 and Hyal2 are expressed by satellite cells (132), suggesting that these cells directly contribute to HA synthesis in muscle.

When muscle is damaged (*e.g.* via toxin injection, mechanical overload), HA content as well as Has1, Has2 and Hyal2 expression greatly increased in satellite cells and other muscle-resident cells (132; 133). This increase in HA is thought to promote myoblast proliferation (134-136) and migration (134-137). HA also inhibited fusion (134; 137), which was mediated by binding to HA through CD44 and/or RHAMM (136-138). The activation of Has2 in damaged muscle was recently shown to be driven by histone demethylation (via JMJD3), which enabled satellite cells to re-enter the cell cycle (139).

In developing muscle, expression of HA is initially high, which is thought to promote myogenic progenitor migration *in vivo* (140). Has2 and Has3 were upregulated during muscle development (133; 136), and knockdown of Has2 negatively affected muscle cell migration *in vivo* (138), mediated by CD44 (136; 138). During subsequent differentiation, the abundance of HA decreased around differentiating muscle *in vivo* (134; 136) and *in vitro* (141), suggesting that HA negatively influences differentiation. These observations are supported by *in vitro* studies in which exogenously supplied HA, either via substrate coating or in the media, inhibited differentiation (134; 142-145). In contrast, knockdown of Has2 in C2C12 cells negatively affected differentiation (133). This discrepancy may be due to differences in exogenously vs endogenously applied HA and the model system used (*e.g.* cell line vs. primary cells, *in vivo* vs.

in vitro). Nevertheless, HA appears to facilitate the increase in myogenic progenitor number to generate adequate cells to form/restore myofibers.

In contrast, an increase in HA may be indicative of a disease state. In insulin-resistant mice (induced by a high fat diet), HA and CD44 content increased, while insulin resistance could be reversed by systemic treatment with intravenous pegylated recombinant PH20 (146). When CD44 was knocked out concomitant with a high fat diet, muscle no longer developed insulin resistance, indicating the interaction of HA with CD44 mediated insulin resistance (147). An increase in HA was also observed in response to stroke, which was hypothesized to increase the passive stiffness of muscle (148).

2.6 Intervertebral Disc and Other Fibrocartilages

Like articular cartilage, fibrocartilaginous tissues such as the intervertebral disc (IVD), menisci, and labrum serve as important load-bearing connective tissues in the body. The IVD is made up of the annulus fibrosus (AF), which has concentric rings (lamellae) of ligament-like tissue and surrounds the hydrogel-like nucleus pulposus (NP). The organization of the AF and NP enable the IVD to facilitate movement of the vertebral elements in torsion, tension, and compression. HA content is highest in the NP (149), and the concentration of HA and other GAGs increases from the cervical to lumbar discs (150). PGs, such as aggrecan, bound to HA maintain the high water content needed in the NPs to resist compression. At the onset of IVD formation, there is little HA, but the content increases over development (150; 151). Hydrostatic pressure may influence HA synthesis as it was shown that *in vitro* stimulation of bovine NP cells increased expression of HAS2 (and other chondrogenic genes) over controls (152). Furthermore, CD44 is hypothesized to play a role in anchoring HA to cells during IVD formation (153).

When HA deposition was disrupted via knockout of Has2 in type II collagen-expressing cells, the initial formation of the IVD was not perturbed, but substantially affected further development (154). Vertebrae were affected as well in this model; however, endochondral ossification still occurred (154). While the IVD progenitors remained when Has2 was knocked out and aggrecan was still synthesized (154), the absence of HA likely affected the retention of aggrecan into a cohesive matrix, which was dependent on HA and Has2 (68).

The cells from the AF and NP appear to respond to HA differently. HA stimulation of human AF cells *in vitro* did not induce chondrogenic genes like type II collagen (COL2A1) and aggrecan but did promote the upregulation of type X collagen (COL10A1) and matrix metalloproteinase (MMP) 13 (155). When TGFβ was included, COL2A1 and COL10A1 were significantly downregulated in AF cells (156). In contrast, HA treatment of NP cells slightly increased overall PG and type II collagen gene expression (157).

During disc degeneration, high molecular weight HA was thought to suppress the inflammatory response and nerve growth factor and brain-derived neurotrophic factor gene expression in bovine NP cells (158), and HAS2 was upregulated in AF cells from degenerated human discs compared to controls (159), suggesting HA is beneficial. However, expression of HYAL1, HYAL2, HYAL3 and CEMIP were also reported to increase in IVD disease (160; 161), where HYAL2 is hypothesized to be the most relevant hyaluronidase (161).

3 Hyaluronan in Mechanotransduction and Mechanobiology

As detailed above, the structural and mechanical role of HA in synovial fluid and articular cartilage is well described and mediated through interactions with PGs. In bone, tendon, ligament, muscle, and other tissues, the direct contribution of HA to mechanics is less clear, although the absence or reduction of HA is associated with changes to structure-function relationships. While the overall physical properties of HA will likely be similar in other tissues, it is apparent that the exact function of this molecule is highly context-dependent and thus its behavior may differ among different musculoskeletal tissues. Nevertheless, HA's structural role in synovial fluid and musculoskeletal connective tissues links it to multi-scale synovial joint function and mechanics and implies a direct role in transduction of tissue-scale mechanical loading into mechanoresponsive cell behaviors.

Efforts to design biomaterials for tissue engineering often seek to mimic the native ECM, including its component biomolecules and their complex interactions (4), to recapitulate both the mechanical behavior and the signaling environment encountered by resident cells. With the growing appreciation of mechanobiology in the maturation, integration, and maintenance of repair and regenerated tissues, biomedical engineering of HA-rich tissues must also consider the roles of HA both in driving mechanical behavior and in response to altered mechanics (2). We consider both how HA in the ECM affects the transduction of mechanical cues and how mechanical cues alter HA metabolism.

3.1 Hyaluronan Modulation of Mechanotransduction

The HA-rich glycocalyx or pericellular coat of cells plays a role in transducing mechanical cues from the ECM to the cell (61; 162). As noted above, each HAS isoform produces HA of varying molecular weight and localization on the cell surface (7; 8) as well as differential expression among different cell types (9). The localization of HA at the PCM influences the transmission of external force in various ways. For example, plasma membrane protrusions into the HA-rich pericellular coat (163) suggest a potential interface by which mechanical cues can be transduced through the glycocalyx into the cell. In the joint, inhibition of p38 MAPK inhibited assembly of HA-rich PCM during development via regulation of MEK-ERK pathway in a manner specific to cyclic uniaxial loading (164). Similarly, removal of the HA-rich glycocalyx from myoblasts and myotubes *in vitro* inhibited the ability of C2C12 myotubes to generate nitric oxide in response to shear stress (165).

The binding of HA with adjacent ECM components, receptors, and other molecules may also play a role in mechanotransduction, although we are not aware of any studies directly probing these interactions in musculoskeletal tissues. Hyaluronan-CD44 interactions that inhibit osteocyte migration and cell-cell communication (106) are thought to affect the transmission of mechanical cues from the ECM to osteocytes. Because strong expression of CD44 is found in osteocyte lacunae (19; 20; 166), pericellular HA content and localization likely modulates osteocyte mechanobiology. HA may also directly influence cell-matrix interactions, a premise suggested by evidence that HA can augment integrin-mediated mechanotransduction (167). Indeed, a soft hydrogel with both HA and type I collagen mimicked the effect of a stiff substrate, resulting in the generation of stress fibers and large focal adhesions (168). Although these observations were made in a hepatocyte cell line, similar mechanisms may be behind observations that introduction of HA alters cell and tissue responses to load in musculoskeletal tissues. For example, HA suppressed mechanical stress-induced expression of ADAMTS4,

ADAMTS5, MMP13 in human articular chondrocytes exposed to cyclic tensile strain at 10%, 0.5 Hz (169).

3.2 Mechanoregulation of Hyaluronan and Mechanobiological Interactions

Mechanical loading modulates endogenous production of HA; an early study showed joint immobilization in sheep reduced HA concentration within synovial fluid (170) whereas cyclic movement stimulated HA secretion (171; 172). Supporting these *in vivo* observations, primary rabbit synoviocytes increase production of HA under static stretch (173) via Ca^{2+} dependent PKCα-MAPK signaling pathway (174). Cyclic compression upregulated *HAS2* and *HAS3* in synoviocytes embedded within a collagen gel (175), and *in vitro* stimulation of bovine NP cells increased expression of HAS2 and other chondrogenic genes (152).

Mechanical cues also modulate the expression and activity of HA catabolic enzymes. Cyclic tensile stretch of over 20% in rabbit synovial cells increased HYAL1 and HYAL2 gene expression, with enzymatic activity detectable in loaded but not unloaded cells (47). In a human synoviocyte cell line, strain-level dependent expression changes in *HYAL1*, *HYAL2*, and *TMEM* were observed alongside net decreases in HA content in the cell medium and increased intracellular staining for HA (176), indicative of HA reuptake for breakdown (177). Excessive tension of cultured chondrocytes led to significant upregulation of HYAL1, HYAL2, and IL-1β expression and moderate increases in TNFα (178).

3.3 Potential Interactions with Mechanical Loading

Mechanical loading may also modulate the effects of other known factors that regulate HA synthesis and catabolic enzyme activity. Because few studies directly address potential interactions between mechanobiology and HA metabolism, we provide instead of a brief overview of potential factors. Oguchi and others found that inflammatory cytokines can be used to differentially stimulate HAS activity (179). Has1 requires higher glucosamine (180) and glucose (181) concentrations or the presence of inflammatory signaling (i.e., IL-1β, TNFα, TGFβ) to produce a robust, CD44-dependent hyaluronan coat (181). As noted above, both CD44 interactions and the HA coat play roles in mechanotransduction. In addition, a higher cellular content of uridine diphosphate (UDP)-GlcNAc precursors was required for HAS1 activity compared to HAS2 and HAS3 (180). Increasing UDP-GlcNAc availability alone was advantageous for HA synthesis but only up to a point (182), as there appear to be other yet-unknown factors that contribute to control of HA molecular weight. Given the relationship between biomechanics and ECM remodeling, these unknown factors likely include cell-cell and cell-matrix interactions and substrate stiffness.

Because of the association with HA content and disease, manipulation of HA content in synovial fluid has been used as a therapeutic tool to alter joint mechanics. Exogenous administration of HA into the synovial joint – often referred to as viscosupplementation – has been associated with improvements to lubrication and reduction of inflammation and fibrosis (183-187), albeit short-lived due in part to the fast clearance of HA from the joint (188). Furthermore, several meta-analyses of randomized controlled trials of HA injections as a therapy for knee pain or OA suggest that current treatment approaches are not clinically effective compared to placebo or other injectable therapies, and in fact introduce a risk for adverse events such as joint inflammation response following the treatment (189; 190). Thus, a better understanding of HA structure, properties, and interactions with other molecules have the potential to assist the development of new intra-articular therapies for viscosupplementation (2).

For example, increasing expression of *HAS2* has been shown to increase HA concentrations (191; 192), suggesting that promoting endogenous HA production – potentially by enhancing mechanobiological signaling – may be a more effective long-term solution than injection of exogenous HA.

4 Future Issues

Despite the ubiquity of HA in many tissues of the body and the importance of ECM content to musculoskeletal tissue mechanics and mechanobiology, there remain significant knowledge gaps in the underlying biology and mechanobiology of HA metabolism in musculoskeletal tissues. For example, the distribution of hyaluronidases and HA receptors beyond CD44 remain unknown in tendons. These gaps in our understanding of the fundamental workings of HA impair the biomedical engineer in using HA as a biomaterial, designing optimal strategies for tissue repair and regeneration, and directly manipulating HA content and interactions. Little is known in musculoskeletal tissues about the mechanisms by which mechanobiological pathways may govern HA metabolism in musculoskeletal health and orthopedic disease, nor how these mechanisms may interact with disease-related factors such as injury, inflammation, and aging. The roles of physical cues such as substrate stiffness and cyclic mechanical loading in driving HA synthesis, hyaluronidase activity, and inhibition of hyaluronidase – and their associated mechanisms – remains unclear. Despite the importance of HA in healthy tissues and tissue engineering, much remains to be uncovered about the factors that maintain homeostatic levels of HA in healthy musculoskeletal tissues and those may upset the balance between HA synthesis and degradation and between hyaluronidases and their inhibitors.

5 Summary

HA plays a critical role in multiple musculoskeletal tissues and biofluids. However, its function and properties are relatively understudied, particularly outside of its roles in articular cartilage and synovial fluid. Beyond mechanobiology and therapeutics, this review has shown that there is much room for growth in our basic understanding of the role of HA content and metabolism in bone, tendon, ligament, and fibrocartilage biomechanics. A more thorough understanding of the structure-function relationships of HA with other ECM molecules, as well as its multiple roles in regulating cell behavior, will provide important insights into the development of new therapies for musculoskeletal conditions.

6 Literature Cited

1. Cowman MK, Lee HG, Schwertfeger KL, McCarthy JB, Turley EA. 2015. The Content and Size of Hyaluronan in Biological Fluids and Tissues. *Front Immunol* 6:261
2. Kim YS, Guilak F. 2022. Engineering Hyaluronic Acid for the Development of New Treatment Strategies for Osteoarthritis. *International journal of molecular sciences* 23

3. Decker B, Bosch U, Gassler N, Tugtekin I, Kasperczyk W, Reale E. 1994. Histochemical aspects of the proteoglycans of patellar tendon autografts used to replace the posterior cruciate ligament. *Matrix Biol* 14:101-11
4. Tonti OR, Larson H, Lipp SN, Luetkemeyer CM, Makam M, et al. 2021. Tissue-specific parameters for the design of ECM-mimetic biomaterials. *Acta Biomater* 132:83-102
5. Bost F, Diarra-Mehrpour M, Martin JP. 1998. Inter-alpha-trypsin inhibitor proteoglycan family--a group of proteins binding and stabilizing the extracellular matrix. *Eur J Biochem* 252:339-46
6. Itano N, Sawai T, Yoshida M, Lenas P, Yamada Y, et al. 1999. Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. *J Biol Chem* 274:25085-92
7. Itano N, Atsumi F, Sawai T, Yamada Y, Miyaishi O, et al. 2002. Abnormal accumulation of hyaluronan matrix diminishes contact inhibition of cell growth and promotes cell migration. *Proc Natl Acad Sci U S A* 99:3609-14
8. Torronen K, Nikunen K, Karna R, Tammi M, Tammi R, Rilla K. 2014. Tissue distribution and subcellular localization of hyaluronan synthase isoenzymes. *Histochem Cell Biol* 141:17-31
9. Recklies AD, White C, Melching L, Roughley PJ. 2001. Differential regulation and expression of hyaluronan synthases in human articular chondrocytes, synovial cells and osteosarcoma cells. *Biochem J* 354:17-24
10. Itano N, Kimata K. 2002. Mammalian hyaluronan synthases. *IUBMB Life* 54:195-9
11. Laurent TC, Laurent UB, Fraser JR. 1996. The structure and function of hyaluronan: An overview. *Immunol Cell Biol* 74:A1-7
12. Saari H, Sorsa T, Konttinen YT. 1990. Reactive oxygen species and hyaluronate in serum and synovial fluid in arthritis. *Int J Tissue React* 12:81-9
13. Fuchs B, Schiller J. 2014. Glycosaminoglycan degradation by selected reactive oxygen species. *Antioxid Redox Signal* 21:1044-62
14. Stern R, Jedrzejas MJ. 2006. Hyaluronidases: their genomics, structures, and mechanisms of action. *Chem Rev* 106:818-39
15. Csoka AB, Frost GI, Stern R. 2001. The six hyaluronidase-like genes in the human and mouse genomes. *Matrix Biol* 20:499-508
16. Lesley J, Hascall VC, Tammi M, Hyman R. 2000. Hyaluronan binding by cell surface CD44. *J Biol Chem* 275:26967-75
17. Turley EA. 1982. Purification of a hyaluronate-binding protein fraction that modifies cell social behavior. *Biochemical and biophysical research communications* 108:1016-24
18. Henderson KJ, Edwards JC, Worrall JG. 1994. Expression of CD44 in normal and rheumatoid synovium and cultured synovial fibroblasts. *Ann Rheum Dis* 53:729-34
19. Nakamura H, Kenmotsu S, Sakai H, Ozawa H. 1995. Localization of CD44, the hyaluronate receptor, on the plasma membrane of osteocytes and osteoclasts in rat tibiae. *Cell Tissue Res* 280:225-33
20. Noonan KJ, Stevens JW, Tammi R, Tammi M, Hernandez JA, Midura RJ. 1996. Spatial distribution of CD44 and hyaluronan in the proximal tibia of the growing rat. *J Orthop Res* 14:573-81
21. Suzuki A, Nozawa-Inoue K, Amizuka N, Ono K, Maeda T. 2006. Localization of CD44 and hyaluronan in the synovial membrane of the rat temporomandibular joint. *Anat Rec A Discov Mol Cell Evol Biol* 288:646-52

22. Knudson CB. 2003. Hyaluronan and CD44: strategic players for cell-matrix interactions during chondrogenesis and matrix assembly. *Birth defects research. Part C, Embryo today : reviews* 69:174-96
23. Messam BJ, Tolg C, McCarthy JB, Nelson AC, Turley EA. 2021. RHAMM Is a Multifunctional Protein That Regulates Cancer Progression. *International journal of molecular sciences* 22
24. Guilak F, Nims RJ, Dicks A, Wu CL, Meulenbelt I. 2018. Osteoarthritis as a disease of the cartilage pericellular matrix. *Matrix Biol* 71-72:40-50
25. Barland P, Novikoff AB, Hamerman D. 1962. Electron microscopy of the human synovial membrane. *J Cell Biol* 14:207-20
26. Edwards JC. 1994. The nature and origins of synovium: experimental approaches to the study of synoviocyte differentiation. *Journal of anatomy* 184 (Pt 3):493-501
27. Smith MD. 2011. The normal synovium. *Open Rheumatol J* 5:100-6
28. Balazs EA, Watson D, Duff IF, Roseman S. 1967. Hyaluronic acid in synovial fluid. I. Molecular parameters of hyaluronic acid in normal and arthritis human fluids. *Arthritis Rheum* 10:357-76
29. Dahl LB, Dahl IM, Engstrom-Laurent A, Granath K. 1985. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. *Ann Rheum Dis* 44:817-22
30. Temple-Wong MM, Ren S, Quach P, Hansen BC, Chen AC, et al. 2016. Hyaluronan concentration and size distribution in human knee synovial fluid: variations with age and cartilage degeneration. *Arthritis Res Ther* 18:18
31. Schmidt TA, Gastelum NS, Nguyen QT, Schumacher BL, Sah RL. 2007. Boundary lubrication of articular cartilage: role of synovial fluid constituents. *Arthritis Rheum* 56:882-91
32. Coleman PJ, Scott D, Ray J, Mason RM, Levick JR. 1997. Hyaluronan secretion into the synovial cavity of rabbit knees and comparison with albumin turnover. *The Journal of Physiology* 503 (Pt 3):645-56
33. Sabaratnam S, Arunan V, Coleman PJ, Mason RM, Levick JR. 2005. Size selectivity of hyaluronan molecular sieving by extracellular matrix in rabbit synovial joints. *The Journal of Physiology* 567:569-81
34. Yanagida-Suekawa T, Tanimoto K, Tanne Y, Mitsuyoshi T, Hirose N, et al. 2013. Synthesis of hyaluronan and superficial zone protein in synovial membrane cells modulated by fluid flow. *Eur J Oral Sci* 121:566-72
35. Schumacher BL, Block JA, Schmid TM, Aydelotte MB, Kuettner KE. 1994. A novel proteoglycan synthesized and secreted by chondrocytes of the superficial zone of articular cartilage. *Archives of biochemistry and biophysics* 311:144-52
36. Greene GW, Banquy X, Lee DW, Lowrey DD, Yu J, Israelachvili JN. 2011. Adaptive mechanically controlled lubrication mechanism found in articular joints. *Proc Natl Acad Sci U S A* 108:5255-9
37. Bonnevie ED, Galesso D, Secchieri C, Cohen I, Bonassar LJ. 2015. Elastoviscous Transitions of Articular Cartilage Reveal a Mechanism of Synergy between Lubricin and Hyaluronic Acid. *PLoS One* 10:e0143415
38. Chang DP, Abu-Lail NI, Coles JM, Guilak F, Jay GD, Zauscher S. 2009. Friction Force Microscopy of Lubricin and Hyaluronic Acid between Hydrophobic and Hydrophilic Surfaces. *Soft Matter* 5:3438-45

39. Chang DP, Abu-Lail NI, Guilak F, Jay GD, Zauscher S. 2008. Conformational mechanics, adsorption, and normal force interactions of lubricin and hyaluronic acid on model surfaces. *Langmuir* 24:1183-93
40. Ludwig TE, Cowman MK, Jay GD, Schmidt TA. 2014. Effects of concentration and structure on proteoglycan 4 rheology and interaction with hyaluronan. *Biorheology* 51:409-22
41. Lin W, Liu Z, Kampf N, Klein J. 2020. The Role of Hyaluronic Acid in Cartilage Boundary Lubrication. *Cells* 9
42. Tanimoto K, Ohno S, Fujimoto K, Honda K, Ijuin C, et al. 2001. Proinflammatory cytokines regulate the gene expression of hyaluronic acid synthetase in cultured rabbit synovial membrane cells. *Connect Tissue Res* 42:187-95
43. Uitterlinden EJ, Koevoet JL, Verkoelen CF, Bierma-Zeinstra SM, Jahr H, et al. 2008. Glucosamine increases hyaluronic acid production in human osteoarthritic synovium explants. *BMC Musculoskelet Disord* 9:120
44. Mustonen AM, Nieminen P, Joukainen A, Jaroma A, Kaariainen T, et al. 2016. First in vivo detection and characterization of hyaluronan-coated extracellular vesicles in human synovial fluid. *J Orthop Res* 34:1960-8
45. Brown TJ, Laurent UB, Fraser JR. 1991. Turnover of hyaluronan in synovial joints: elimination of labelled hyaluronan from the knee joint of the rabbit. *Exp Physiol* 76:125-34
46. Lindenhayn K, Heilmann HH, Niederhausen T, Walther HU, Pohlenz K. 1997. Elimination of tritium-labelled hyaluronic acid from normal and osteoarthritic rabbit knee joints. *Eur J Clin Chem Clin Biochem* 35:355-63
47. Kitamura R, Tanimoto K, Tanne Y, Kamiya T, Huang YC, et al. 2010. Effects of mechanical load on the expression and activity of hyaluronidase in cultured synovial membrane cells. *Journal of biomedical materials research. Part A* 92:87-93
48. Wu J, Qu Y, Zhang YP, Deng JX, Yu QH. 2018. RHAMM induces progression of rheumatoid arthritis by enhancing the functions of fibroblast-like synoviocytes. *BMC Musculoskelet Disord* 19:455
49. Dunn S, Kolomytkin OV, Waddell DD, Marino AA. 2009. Hyaluronan-binding receptors: possible involvement in osteoarthritis. *Modern rheumatology / the Japan Rheumatism Association* 19:151-5
50. Band PA, Heeter J, Wisniewski HG, Liublinska V, Pattanayak CW, et al. 2015. Hyaluronan molecular weight distribution is associated with the risk of knee osteoarthritis progression. *Osteoarthritis Cartilage* 23:70-6
51. Peal BT, Gagliardi R, Su J, Fortier LA, Delco ML, et al. 2020. Synovial fluid lubricin and hyaluronan are altered in equine osteochondral fragmentation, cartilage impact injury, and full-thickness cartilage defect models. *J Orthop Res* 38:1826-35
52. McCarty WJ, Cheng JC, Hansen BC, Yamaguchi T, Firestein GS, et al. 2012. The biophysical mechanisms of altered hyaluronan concentration in synovial fluid after anterior cruciate ligament transection. *Arthritis Rheum* 64:3993-4003
53. Ludwig TE, McAllister JR, Lun V, Wiley JP, Schmidt TA. 2012. Diminished cartilage-lubricating ability of human osteoarthritic synovial fluid deficient in proteoglycan 4: Restoration through proteoglycan 4 supplementation. *Arthritis Rheum* 64:3963-71

54. Lambert C, Dubuc JE, Montell E, Verges J, Munaut C, et al. 2014. Gene expression pattern of cells from inflamed and normal areas of osteoarthritis synovial membrane. *Arthritis Rheumatol* 66:960-8
55. Nagaya H, Ymagata T, Ymagata S, Iyoda K, Ito H, et al. 1999. Examination of synovial fluid and serum hyaluronidase activity as a joint marker in rheumatoid arthritis and osteoarthritis patients (by zymography). *Ann Rheum Dis* 58:186-8
56. Yoshida M, Sai S, Marumo K, Tanaka T, Itano N, et al. 2004. Expression analysis of three isoforms of hyaluronan synthase and hyaluronidase in the synovium of knees in osteoarthritis and rheumatoid arthritis by quantitative real-time reverse transcriptase polymerase chain reaction. *Arthritis Res Ther* 6:R514-20
57. Shiozawa J, de Vega S, Cilek MZ, Yoshinaga C, Nakamura T, et al. 2020. Implication of HYBID (Hyaluronan-Binding Protein Involved in Hyaluronan Depolymerization) in Hyaluronan Degradation by Synovial Fibroblasts in Patients with Knee Osteoarthritis. *Am J Pathol* 190:1046-58
58. Deroyer C, Poulet C, Paulissen G, Ciregia F, Malaise O, et al. 2022. CEMIP (KIAA1199) regulates inflammation, hyperplasia and fibrosis in osteoarthritis synovial membrane. *Cell Mol Life Sci* 79:260
59. Atarod M, Ludwig TE, Frank CB, Schmidt TA, Shrive NG. 2015. Cartilage boundary lubrication of ovine synovial fluid following anterior cruciate ligament transection: a longitudinal study. *Osteoarthritis Cartilage* 23:640-7
60. Poole CA. 1997. Articular cartilage chondrons: form, function and failure. *Journal of anatomy* 191 (Pt 1):1-13
61. Knudson W, Ishizuka S, Terabe K, Askew EB, Knudson CB. 2019. The pericellular hyaluronan of articular chondrocytes. *Matrix Biol* 78-79:32-46
62. Lai WM, Hou JS, Mow VC. 1991. A triphasic theory for the swelling and deformation behaviors of articular cartilage. *J Biomech Eng* 113:245-58
63. Buschmann MD, Grodzinsky AJ. 1995. A molecular model of proteoglycan-associated electrostatic forces in cartilage mechanics. *J Biomech Eng* 117:179-92
64. Ateshian GA. 2009. The role of interstitial fluid pressurization in articular cartilage lubrication. *J Biomech* 42:1163-76
65. Ohtsuki T, Asano K, Inagaki J, Shinaoka A, Kumagishi-Shinaoka K, et al. 2018. High molecular weight hyaluronan protects cartilage from degradation by inhibiting aggrecanase expression. *J Orthop Res* 36:3247-55
66. Plaas A, Osborn B, Yoshihara Y, Bai Y, Bloom T, et al. 2007. Aggrecanolysis in human osteoarthritis: confocal localization and biochemical characterization of ADAMTS5-hyaluronan complexes in articular cartilages. *Osteoarthritis Cartilage* 15:719-34
67. Kataoka Y, Ariyoshi W, Okinaga T, Kaneuji T, Mitsugi S, et al. 2013. Mechanisms involved in suppression of ADAMTS4 expression in synoviocytes by high molecular weight hyaluronic acid. *Biochemical and biophysical research communications* 432:580-5
68. Huang Y, Askew EB, Knudson CB, Knudson W. 2016. CRISPR/Cas9 knockout of HAS2 in rat chondrosarcoma chondrocytes demonstrates the requirement of hyaluronan for aggrecan retention. *Matrix Biol* 56:74-94
69. Hiscock DR, Caterson B, Flannery CR. 2000. Expression of hyaluronan synthases in articular cartilage. *Osteoarthritis Cartilage* 8:120-6

70. Nishida Y, Knudson CB, Nietfeld JJ, Margulis A, Knudson W. 1999. Antisense inhibition of hyaluronan synthase-2 in human articular chondrocytes inhibits proteoglycan retention and matrix assembly. *J Biol Chem* 274:21893-9
71. Ohno S, Tanimoto K, Fujimoto K, Ijuin C, Honda K, et al. 2001. Molecular cloning of rabbit hyaluronic acid synthases and their expression patterns in synovial membrane and articular cartilage. *Biochim Biophys Acta* 1520:71-8
72. Albrecht C, Schlegel W, Eckl P, Jagersberger T, Sadeghi K, et al. 2009. Alterations in CD44 isoforms and HAS expression in human articular chondrocytes during the de- and re-differentiation processes. *Int J Mol Med* 23:253-9
73. Ono Y, Sakai T, Hiraiwa H, Hamada T, Omachi T, et al. 2013. Chondrogenic capacity and alterations in hyaluronan synthesis of cultured human osteoarthritic chondrocytes. *Biochemical and biophysical research communications* 435:733-9
74. Chow G, Knudson CB, Knudson W. 2006. Human hyaluronidase-2 is localized intracellularly in articular chondrocytes and other cultured cell lines. *Osteoarthritis Cartilage* 14:1312-4
75. Tanimoto K, Suzuki A, Ohno S, Honda K, Tanaka N, et al. 2004. Hyaluronidase expression in cultured growth plate chondrocytes during differentiation. *Cell Tissue Res* 318:335-42
76. Shimoda M, Yoshida H, Mizuno S, Hirozane T, Horiuchi K, et al. 2017. Hyaluronan-Binding Protein Involved in Hyaluronan Depolymerization Controls Endochondral Ossification through Hyaluronan Metabolism. *Am J Pathol* 187:1162-76
77. Higuchi Y, Nishida Y, Kozawa E, Zhuo L, Arai E, et al. 2017. Conditional knockdown of hyaluronidase 2 in articular cartilage stimulates osteoarthritic progression in a mice model. *Sci Rep* 7:7028
78. Chow G, Knudson CB, Knudson W. 2006. Expression and cellular localization of human hyaluronidase-2 in articular chondrocytes and cultured cell lines. *Osteoarthritis Cartilage* 14:849-58
79. Miller AD, Vigdorovich V, Strong RK, Fernandes RJ, Lerman MI. 2006. Hyal2, where are you? *Osteoarthritis Cartilage* 14:1315-7
80. Hida D, Danielson BT, Knudson CB, Knudson W. 2015. CD44 knock-down in bovine and human chondrocytes results in release of bound HYAL2. *Matrix Biol* 48:42-54
81. Iacob S, Knudson CB. 2006. Hyaluronan fragments activate nitric oxide synthase and the production of nitric oxide by articular chondrocytes. *Int J Biochem Cell Biol* 38:123-33
82. Knudson CB. 1993. Hyaluronan receptor-directed assembly of chondrocyte pericellular matrix. *J Cell Biol* 120:825-34
83. Knudson W, Casey B, Nishida Y, Eger W, Kuettner KE, Knudson CB. 2000. Hyaluronan oligosaccharides perturb cartilage matrix homeostasis and induce chondrocytic chondrolysis. *Arthritis Rheum* 43:1165-74
84. Ishida O, Tanaka Y, Morimoto I, Takigawa M, Eto S. 1997. Chondrocytes are regulated by cellular adhesion through CD44 and hyaluronic acid pathway. *J Bone Miner Res* 12:1657-63
85. Ding QH, Qi YY, Li XM, Chen WP, Wang XH, Ji XW. 2019. Knockdown of KIAA1199 suppresses IL-1beta-induced cartilage degradation and inflammatory responses in human chondrocytes through the Wnt/beta-catenin signalling pathway. *Int Immunopharmacol* 73:203-11

86. Deroyer C, Charlier E, Neuville S, Malaise O, Gillet P, et al. 2019. CEMIP (KIAA1199) induces a fibrosis-like process in osteoarthritic chondrocytes. *Cell Death Dis* 10:103
87. Shimizu H, Shimoda M, Mochizuki S, Miyamae Y, Abe H, et al. 2018. Hyaluronan-Binding Protein Involved in Hyaluronan Depolymerization Is Up-Regulated and Involved in Hyaluronan Degradation in Human Osteoarthritic Cartilage. *Am J Pathol* 188:2109-19
88. Shiozawa J, de Vega S, Yoshinaga C, Ji X, Negishi Y, et al. 2022. Expression and regulation of recently discovered hyaluronidases, HYBID and TMEM2, in chondrocytes from knee osteoarthritic cartilage. *Sci Rep* 12:17242
89. Boskey AL. 2013. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. *Bonekey Rep* 2:447
90. Bastow ER, Byers S, Golub SB, Clarkin CE, Pitsillides AA, Fosang AJ. 2008. Hyaluronan synthesis and degradation in cartilage and bone. *Cell Mol Life Sci* 65:395-413
91. Pavasant P, Shizari T, Underhill CB. 1996. Hyaluronan contributes to the enlargement of hypertrophic lacunae in the growth plate. *J Cell Sci* 109 (Pt 2):327-34
92. Moffatt P, Lee ER, St-Jacques B, Matsumoto K, Yamaguchi Y, Roughley PJ. 2011. Hyaluronan production by means of Has2 gene expression in chondrocytes is essential for long bone development. *Dev Dyn* 240:404-12
93. Takada Y, Sakiyama H, Kuriiwa K, Masuda R, Inoue N, et al. 1999. Metabolic activities of partially degenerated hypertrophic chondrocytes: gene expression of hyaluronan synthases. *Cell Tissue Res* 298:317-25
94. Pendyala M, Stephen SJ, Vashishth D, Blaber EA, Chan DD. 2023. Loss of hyaluronan synthases impacts bone morphology, quality, and mechanical properties. *Bone* 172:116779
95. Oohira A, Nogami H. 1989. Elevated accumulation of hyaluronate in the tubular bones of osteogenesis imperfecta. *Bone* 10:409-13
96. Imbert L, Auregan JC, Pernelle K, Hoc T. 2014. Mechanical and mineral properties of osteogenesis imperfecta human bones at the tissue level. *Bone* 65:18-24
97. Adams JR, Sander G, Byers S. 2006. Expression of hyaluronan synthases and hyaluronidases in the MG63 osteoblast cell line. *Matrix Biol* 25:40-6
98. Huang L, Cheng YY, Koo PL, Lee KM, Qin L, et al. 2003. The effect of hyaluronan on osteoblast proliferation and differentiation in rat calvarial-derived cell cultures. *Journal of biomedical materials research. Part A* 66:880-4
99. Luben RA, Cohn DV. 1976. Effects of parathormone and calcitonin on citrate and hyaluronate metabolism in cultured bone. *Endocrinology* 98:413-9
100. Luben RA, Goggins JF, Raisz LG. 1974. Stimulation by parathyroid hormone of bone hyaluronate synthesis in organ culture. *Endocrinology* 94:737-45
101. Severson AR. 1979. Colchicine stimulation of hyaluronate synthesis and secretion in bone organ culture. *Journal of cellular physiology* 101:341-8
102. Prince CW. 2004. Roles of hyaluronan in bone resorption. *BMC Musculoskelet Disord* 5:12
103. Chen L, Shi K, Andersen TL, Qiu W, Kassem M. 2019. KIAA1199 is a secreted molecule that enhances osteoblastic stem cell migration and recruitment. *Cell Death Dis* 10:126

104. Chen L, Shi K, Ditzel N, Qiu W, Figeac F, et al. 2023. KIAA1199 deficiency enhances skeletal stem cell differentiation to osteoblasts and promotes bone regeneration. *Nat Commun* 14:2016
105. Puissant E, Gilis F, Tevel V, Vandeweerd JM, Flamion B, et al. 2022. Hyaluronidase 1 deficiency decreases bone mineral density in mice. *Sci Rep* 12:10142
106. Spessotto P, Rossi FM, Degan M, Di Francia R, Perris R, et al. 2002. Hyaluronan-CD44 interaction hampers migration of osteoclast-like cells by down-regulating MMP-9. *J Cell Biol* 158:1133-44
107. Chang EJ, Kim HJ, Ha J, Kim HJ, Ryu J, et al. 2007. Hyaluronan inhibits osteoclast differentiation via Toll-like receptor 4. *J Cell Sci* 120:166-76
108. Cao JJ, Singleton PA, Majumdar S, Boudignon B, Burghardt A, et al. 2005. Hyaluronan increases RANKL expression in bone marrow stromal cells through CD44. *J Bone Miner Res* 20:30-40
109. Ariyoshi W, Takahashi T, Kanno T, Ichimiya H, Takano H, et al. 2005. Mechanisms involved in enhancement of osteoclast formation and function by low molecular weight hyaluronic acid. *J Biol Chem* 280:18967-72
110. Nakao Y, Hikiji H, Okinaga T, Takeuchi J, Habu M, et al. 2019. Accumulation of hyaluronic acid in stromal cells modulates osteoclast formation by regulation of receptor activator of nuclear factor kappa-B ligand expression. *Biochemical and biophysical research communications* 512:537-43
111. Vogel KG, Peters JA. 2005. Histochemistry defines a proteoglycan-rich layer in bovine flexor tendon subjected to bending. *J Musculoskelet Neuronal Interact* 5:64-9
112. Gillard GC, Reilly HC, Bell-Booth PG, Flint MH. 1979. The influence of mechanical forces on the glycosaminoglycan content of the rabbit flexor digitorum profundus tendon. *Connect Tissue Res* 7:37-46
113. Scott JE, Orford CR, Hughes EW. 1981. Proteoglycan-collagen arrangements in developing rat tail tendon. An electron microscopical and biochemical investigation. *Biochem J* 195:573-81
114. Watanabe M, Nojima M, Shibata T, Hamada M. 1994. Maturation-related biochemical changes in swine anterior cruciate ligament and tibialis posterior tendon. *J Orthop Res* 12:672-82
115. Parry DA, Flint MH, Gillard GC, Craig AS. 1982. A role for glycosaminoglycans in the development of collagen fibrils. *FEBS letters* 149:1-7
116. Scott JE, Hughes EW. 1986. Proteoglycan-collagen relationships in developing chick and bovine tendons. Influence of the physiological environment. *Connect Tissue Res* 14:267-78
117. Miescher I, Wolint P, Opelz C, Snedeker JG, Giovanoli P, et al. 2022. Impact of High-Molecular-Weight Hyaluronic Acid on Gene Expression in Rabbit Achilles Tenocytes In Vitro. *International journal of molecular sciences* 23
118. Wiig M, Abrahamsson SO. 2000. Hyaluronic acid modulates cell proliferation unequally in intrasynovial and extrasynovial rabbit tendons in vitro. *J Hand Surg Br* 25:183-7
119. Yamada T, Gotoh M, Nakama K, Mitsui Y, Higuchi F, Nagata K. 2007. Effects of hyaluronan on cell proliferation and mRNA expression of procollagens alpha 1 (I) and alpha 1 (III) in tendon-derived fibroblasts from patients with rotator cuff disease: an in vitro study. *Am J Sports Med* 35:1870-6

120. Yagi M, Sato N, Mitsui Y, Gotoh M, Hamada T, Nagata K. 2010. Hyaluronan modulates proliferation and migration of rabbit fibroblasts derived from flexor tendon epitenon and endotenon. *The Journal of hand surgery* 35:791-6
121. Gallorini M, Berardi AC, Berardocco M, Gissi C, Maffulli N, et al. 2017. Hyaluronic acid increases tendon derived cell viability and proliferation in vitro: comparative study of two different hyaluronic acid preparations by molecular weight. *Muscles Ligaments Tendons J* 7:208-14
122. Sikes KJ, Renner K, Li J, Grande-Allen KJ, Connell JP, et al. 2018. Knockout of hyaluronan synthase 1, but not 3, impairs formation of the retrocalcaneal bursa. *J Orthop Res* 36:2622-32
123. Berglund M, Hart DA, Wiig M. 2007. The inflammatory response and hyaluronan synthases in the rabbit flexor tendon and tendon sheath following injury. *J Hand Surg Eur Vol* 32:581-7
124. Muljadi PM, Andarawis-Puri N. 2023. Glycosaminoglycans modulate microscale mechanics and viscoelasticity in fatigue injured tendons. *J Biomech* 152:111584
125. Riley GP, Harrall RL, Constant CR, Chard MD, Cawston TE, Hazleman BL. 1994. Glycosaminoglycans of human rotator cuff tendons: changes with age and in chronic rotator cuff tendinitis. *Ann Rheum Dis* 53:367-76
126. Mello ML, de Campos Vidal B. 2003. Experimental tendon repair: glycosaminoglycan arrangement in newly synthesized collagen fibers. *Cell Mol Biol (Noisy-le-grand)* 49:579-85
127. Favata M, Beredjiklian PK, Zgonis MH, Beason DP, Crombleholme TM, et al. 2006. Regenerative properties of fetal sheep tendon are not adversely affected by transplantation into an adult environment. *J Orthop Res* 24:2124-32
128. Ansorge HL, Beredjiklian PK, Soslowsky LJ. 2009. CD44 deficiency improves healing tendon mechanics and increases matrix and cytokine expression in a mouse patellar tendon injury model. *J Orthop Res* 27:1386-91
129. Wu PT, Su WR, Li CL, Hsieh JL, Ma CH, et al. 2019. Inhibition of CD44 induces apoptosis, inflammation, and matrix metalloproteinase expression in tendinopathy. *J Biol Chem* 294:20177-84
130. Yuda Y, Kasashima Y, Kuwano A, Sato K, Hattori S, Arai K. 2013. Active hyaluronidase 2 expression in the granulation tissue formed in the healing process of equine superficial digital flexor tendonitis. *J Vet Med Sci* 75:219-23
131. Goody MF, Sher RB, Henry CA. 2015. Hanging on for the ride: adhesion to the extracellular matrix mediates cellular responses in skeletal muscle morphogenesis and disease. *Dev Biol* 401:75-91
132. Calve S, Isaac J, Gumucio JP, Mendias CL. 2012. Hyaluronic acid, HAS1, and HAS2 are significantly upregulated during muscle hypertrophy. *Am J Physiol Cell Physiol* 303:C577-88
133. Hunt LC, Gorman C, Kintakas C, McCulloch DR, Mackie EJ, White JD. 2013. Hyaluronan synthesis and myogenesis: a requirement for hyaluronan synthesis during myogenic differentiation independent of pericellular matrix formation. *J Biol Chem* 288:13006-21
134. Calve S, Odelberg SJ, Simon HG. 2010. A transitional extracellular matrix instructs cell behavior during muscle regeneration. *Dev Biol* 344:259-71

135. Silva Garcia JM, Panitch A, Calve S. 2019. Functionalization of hyaluronic acid hydrogels with ECM-derived peptides to control myoblast behavior. *Acta Biomater* 84:169-79
136. Leng Y, Abdullah A, Wendt MK, Calve S. 2019. Hyaluronic acid, CD44 and RHAMM regulate myoblast behavior during embryogenesis. *Matrix Biol* 78-79:236-54
137. Mylona E, Jones KA, Mills ST, Pavlath GK. 2006. CD44 regulates myoblast migration and differentiation. *Journal of cellular physiology* 209:314-21
138. Ori M, Nardini M, Casini P, Perris R, Nardi I. 2006. XHas2 activity is required during somitogenesis and precursor cell migration in Xenopus development. *Development* 133:631-40
139. Nakka K, Hachmer S, Mokhtari Z, Kovac R, Bandukwala H, et al. 2022. JMJD3 activated hyaluronan synthesis drives muscle regeneration in an inflammatory environment. *Science* 377:666-9
140. Krenn V, Brand-Saberi B, Wachtler F. 1991. Hyaluronic acid influences the migration of myoblasts within the avian embryonic wing bud. *Am J Anat* 192:400-6
141. Orkin RW, Knudson W, Toole BP. 1985. Loss of hyaluronate-dependent coat during myoblast fusion. *Dev Biol* 107:527-30
142. Elson HF, Ingwall JS. 1980. The cell substratum modulates skeletal muscle differentiation. *J Supramol Struct* 14:313-28
143. Kujawa MJ, Pechak DG, Fiszman MY, Caplan AI. 1986. Hyaluronic acid bonded to cell culture surfaces inhibits the program of myogenesis. *Dev Biol* 113:10-6
144. Kujawa MJ, Tepperman K. 1983. Culturing chick muscle cells on glycosaminoglycan substrates: attachment and differentiation. *Dev Biol* 99:277-86
145. Yoshimura M. 1985. Change of hyaluronic acid synthesis during differentiation of myogenic cells and its relation to transformation of myoblasts by Rous sarcoma virus. *Cell Differ* 16:175-85
146. Kang L, Lantier L, Kennedy A, Bonner JS, Mayes WH, et al. 2013. Hyaluronan accumulates with high-fat feeding and contributes to insulin resistance. *Diabetes* 62:1888-96
147. Hasib A, Hennayake CK, Bracy DP, Bugler-Lamb AR, Lantier L, et al. 2019. CD44 contributes to hyaluronan-mediated insulin resistance in skeletal muscle of high-fat-fed C57BL/6 mice. *Am J Physiol Endocrinol Metab* 317:E973-E83
148. Menon RG, Raghavan P, Regatte RR. 2019. Quantifying muscle glycosaminoglycan levels in patients with post-stroke muscle stiffness using T(1ρ) MRI. *Sci Rep* 9:14513
149. Liu X, Krishnamoorthy D, Lin L, Xue P, Zhang F, et al. 2018. A method for characterising human intervertebral disc glycosaminoglycan disaccharides using liquid chromatography-mass spectrometry with multiple reaction monitoring. *Eur Cell Mater* 35:117-31
150. Scott JE, Bosworth TR, Cribb AM, Taylor JR. 1994. The chemical morphology of age-related changes in human intervertebral disc glycosaminoglycans from cervical, thoracic and lumbar nucleus pulposus and annulus fibrosus. *Journal of anatomy* 184 (Pt 1):73-82
151. Caldeira J, Santa C, Osorio H, Molinos M, Manadas B, et al. 2017. Matrisome Profiling During Intervertebral Disc Development And Ageing. *Sci Rep* 7:11629
152. Vieira F, Kang J, Ferreira L, Mizuno S. 2021. Hydrostatic pressure mimicking diurnal spinal movements maintains anabolic turnover in bovine nucleus pulposus cells in vitro. *Eur Cell Mater* 42:246-63

153. Stevens JW, Kurriger GL, Carter AS, Maynard JA. 2000. CD44 expression in the developing and growing rat intervertebral disc. *Dev Dyn* 219:381-90
154. Roughley PJ, Lamplugh L, Lee ER, Matsumoto K, Yamaguchi Y. 2011. The role of hyaluronan produced by Has2 gene expression in development of the spine. *Spine* 36:E914-20
155. Hegewald AA, Zouhair S, Endres M, Cabraja M, Woiciechowsky C, et al. 2013. Towards biological anulus repair: TGF-beta3, FGF-2 and human serum support matrix formation by human anulus fibrosus cells. *Tissue Cell* 45:68-76
156. Hegewald AA, Cluzel J, Kruger JP, Endres M, Kaps C, Thome C. 2014. Effects of initial boost with TGF-beta 1 and grade of intervertebral disc degeneration on 3D culture of human annulus fibrosus cells. *J Orthop Surg Res* 9:73
157. Haberstroh K, Enz A, Zenclussen ML, Hegewald AA, Neumann K, et al. 2009. Human intervertebral disc-derived cells are recruited by human serum and form nucleus pulposus-like tissue upon stimulation with TGF-beta3 or hyaluronan in vitro. *Tissue Cell* 41:414-20
158. Isa IL, Srivastava A, Tiernan D, Owens P, Rooney P, et al. 2015. Hyaluronic Acid Based Hydrogels Attenuate Inflammatory Receptors and Neurotrophins in Interleukin-1beta Induced Inflammation Model of Nucleus Pulposus Cells. *Biomacromolecules* 16:1714-25
159. Gruber HE, Hoelscher GL, Hanley EN, Jr. 2010. Annulus cells from more degenerated human discs show modified gene expression in 3D culture compared with expression in cells from healthier discs. *The spine journal : official journal of the North American Spine Society* 10:721-7
160. Yamamoto T, Suzuki S, Fujii T, Mima Y, Watanabe K, et al. 2021. Efficacy of hyaluronic acid on intervertebral disc inflammation: An in vitro study using notochordal cell lines and human disc cells. *J Orthop Res* 39:2197-208
161. Krupkova O, Greutert H, Boos N, Lemcke J, Liebscher T, Wuertz-Kozak K. 2020. Expression and activity of hyaluronidases HYAL-1, HYAL-2 and HYAL-3 in the human intervertebral disc. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society* 29:605-15
162. Wilusz RE, Guilak F. 2014. High resistance of the mechanical properties of the chondrocyte pericellular matrix to proteoglycan digestion by chondroitinase, aggrecanase, or hyaluronidase. *J Mech Behav Biomed Mater* 38:183-97
163. Rilla K, Tiihonen R, Kultti A, Tammi M, Tammi R. 2008. Pericellular hyaluronan coat visualized in live cells with a fluorescent probe is scaffolded by plasma membrane protrusions. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* 56:901-10
164. Lewthwaite JC, Bastow ER, Lamb KJ, Blenis J, Wheeler-Jones CP, Pitsillides AA. 2006. A specific mechanomodulatory role for p38 MAPK in embryonic joint articular surface cell MEK-ERK pathway regulation. *J Biol Chem* 281:11011-8
165. Juffer P, Bakker AD, Klein-Nulend J, Jaspers RT. 2014. Mechanical loading by fluid shear stress of myotube glycocalyx stimulates growth factor expression and nitric oxide production. *Cell Biochem Biophys* 69:411-9
166. Hughes DE, Salter DM, Simpson R. 1994. CD44 expression in human bone: a novel marker of osteocytic differentiation. *J Bone Miner Res* 9:39-44

167. Chopra A, Murray ME, Byfield FJ, Mendez MG, Halleluyan R, et al. 2014. Augmentation of integrin-mediated mechanotransduction by hyaluronic acid. *Biomaterials* 35:71-82
168. Mandal K, Raz-Ben Aroush D, Graber ZT, Wu B, Park CY, et al. 2019. Soft Hyaluronic Gels Promote Cell Spreading, Stress Fibers, Focal Adhesion, and Membrane Tension by Phosphoinositide Signaling, Not Traction Force. *ACS Nano* 13:203-14
169. Ozawa M, Nishida K, Yoshida A, Saito T, Harada R, et al. 2015. Hyaluronan suppresses mechanical stress-induced expression of catabolic enzymes by human chondrocytes via inhibition of IL-1beta production and subsequent NF-kappaB activation. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]* 64:243-52
170. Pitsillides AA, Skerry TM, Edwards JC. 1999. Joint immobilization reduces synovial fluid hyaluronan concentration and is accompanied by changes in the synovial intimal cell populations. *Rheumatology* 38:1108-12
171. Ingram KR, Wann AK, Angel CK, Coleman PJ, Levick JR. 2008. Cyclic movement stimulates hyaluronan secretion into the synovial cavity of rabbit joints. *The Journal of Physiology* 586:1715-29
172. Ingram KR, Wann AK, Wingate RM, Coleman PJ, McHale N, Levick JR. 2009. Signal pathways regulating hyaluronan secretion into static and cycled synovial joints of rabbits. *The Journal of Physiology* 587:4361-76
173. Momberger TS, Levick JR, Mason RM. 2005. Hyaluronan secretion by synoviocytes is mechanosensitive. *Matrix Biol* 24:510-9
174. Momberger TS, Levick JR, Mason RM. 2006. Mechanosensitive synoviocytes: a Ca2+ - PKCalpha-MAP kinase pathway contributes to stretch-induced hyaluronan synthesis in vitro. *Matrix Biol* 25:306-16
175. Uehara K, Hara M, Matsuo T, Namiki G, Watanabe M, Nomura Y. 2015. Hyaluronic acid secretion by synoviocytes alters under cyclic compressive load in contracted collagen gels. *Cytotechnology* 67:19-26
176. Pendyala M, Woods PS, Brubaker DK, Blaber EA, Schmidt TA, Chan DD. 2022. Endogenous production of hyaluronan, PRG4, and cytokines is sensitive to cyclic loading in synoviocytes. *PLoS One* 17:e0267921
177. Skandalis SS, Karalis T, Heldin P. 2020. Intracellular hyaluronan: Importance for cellular functions. *Semin Cancer Biol* 62:20-30
178. Tanimoto K, Kitamura R, Tanne Y, Kamiya T, Kunimatsu R, et al. 2010. Modulation of hyaluronan catabolism in chondrocytes by mechanical stimuli. *Journal of biomedical materials research. Part A* 93:373-80
179. Oguchi T, Ishiguro N. 2004. Differential stimulation of three forms of hyaluronan synthase by TGF-beta, IL-1beta, and TNF-alpha. *Connect Tissue Res* 45:197-205
180. Rilla K, Oikari S, Jokela TA, Hyttinen JM, Karna R, et al. 2013. Hyaluronan synthase 1 (HAS1) requires higher cellular UDP-GlcNAc concentration than HAS2 and HAS3. *J Biol Chem* 288:5973-83
181. Siiskonen H, Karna R, Hyttinen JM, Tammi RH, Tammi MI, Rilla K. 2014. Hyaluronan synthase 1 (HAS1) produces a cytokine-and glucose-inducible, CD44-dependent cell surface coat. *Experimental cell research* 320:153-63

182. Chen WY, Marcellin E, Steen JA, Nielsen LK. 2014. The role of hyaluronic acid precursor concentrations in molecular weight control in Streptococcus zooepidemicus. *Mol Biotechnol* 56:147-56
183. Wong BL, Kim SH, Antonacci JM, McIlwraith CW, Sah RL. 2010. Cartilage shear dynamics during tibio-femoral articulation: effect of acute joint injury and tribosupplementation on synovial fluid lubrication. *Osteoarthritis Cartilage* 18:464-71
184. Kanazawa K, Hagiwara Y, Tsuchiya M, Yabe Y, Sonofuchi K, et al. 2015. Preventing effects of joint contracture by high molecular weight hyaluronan injections in a rat immobilized knee model. *Int J Clin Exp Pathol* 8:3426-40
185. Shen Q, Li J, Chan D, Sandy JD, Takeuchi J, et al. 2019. Effect of intra-articular hyaluronan injection on inflammation and bone remodeling in the epiphyses and metaphyses of the knee in a murine model of joint injury. *Am J Transl Res* 11:3280-300
186. Li J, Gorski DJ, Anemaet W, Velasco J, Takeuchi J, et al. 2012. Hyaluronan injection in murine osteoarthritis prevents TGFbeta 1-induced synovial neovascularization and fibrosis and maintains articular cartilage integrity by a CD44-dependent mechanism. *Arthritis Res Ther* 14:R151
187. Plaas A, Li J, Riesco J, Das R, Sandy JD, Harrison A. 2011. Intraarticular injection of hyaluronan prevents cartilage erosion, periarticular fibrosis and mechanical allodynia and normalizes stance time in murine knee osteoarthritis. *Arthritis Res Ther* 13:R46
188. Richardson C, Plaas A, Block JA. 2019. Intra-articular Hyaluronan Therapy for Symptomatic Knee Osteoarthritis. *Rheum Dis Clin North Am* 45:439-51
189. Zhao D, Pan JK, Yang WY, Han YH, Zeng LF, et al. 2021. Intra-Articular Injections of Platelet-Rich Plasma, Adipose Mesenchymal Stem Cells, and Bone Marrow Mesenchymal Stem Cells Associated With Better Outcomes Than Hyaluronic Acid and Saline in Knee Osteoarthritis: A Systematic Review and Network Meta-analysis. *Arthroscopy* 37:2298-314 e10
190. Zhao K, Wen Y, Bunpetch V, Lin J, Hu Y, et al. 2022. Hype or hope of hyaluronic acid for osteoarthritis: Integrated clinical evidence synthesis with multi-organ transcriptomics. *J Orthop Translat* 32:91-100
191. Sussmann M, Sarbia M, Meyer-Kirchrath J, Nusing RM, Schror K, Fischer JW. 2004. Induction of hyaluronic acid synthase 2 (HAS2) in human vascular smooth muscle cells by vasodilatory prostaglandins. *Circ Res* 94:592-600
192. Kim S, Kang BY, Cho SY, Sung DS, Chang HK, et al. 2004. Compound K induces expression of hyaluronan synthase 2 gene in transformed human keratinocytes and increases hyaluronan in hairless mouse skin. *Biochemical and biophysical research communications* 316:348-55

7 Related Resources

1. Altman R, Bedi A, Manjoo A, Niazi F, Shaw P, Mease P. 2019. Anti-Inflammatory Effects of Intra-Articular Hyaluronic Acid: A Systematic Review. Cartilage 10:43-52
2. Bastow ER, Byers S, Golub SB, Clarkin CE, Pitsillides AA, Fosang AJ. 2008. Hyaluronan synthesis and degradation in cartilage and bone. Cell Mol Life Sci 65:395-413

3. Dicker KT, Gurski LA, Pradhan-Bhatt S, Witt RL, Farach-Carson MC, Jia X. 2014. Hyaluronan: a simple polysaccharide with diverse biological functions. Acta Biomater 10:1558-70
4. Mio K, Stern R. 2002. Inhibitors of the hyaluronidases. Matrix Biol 21:31-7
5. Misra S, Hascall VC, Markwald RR, Ghatak S. 2015. Interactions between Hyaluronan and Its Receptors (CD44, RHAMM) Regulate the Activities of Inflammation and Cancer. Front Immunol 6:201
6. Stern R, Jedrzejas MJ. 2006. Hyaluronidases: their genomics, structures, and mechanisms of action. Chem Rev 106:818-39
7. Triggs-Raine B, Natowicz MR. 2015. Biology of hyaluronan: Insights from genetic disorders of hyaluronan metabolism. World J Biol Chem 6:110-20

8 Key Terms and Definitions List

Abbreviation	Definition
CD44	cluster of differentiation 44
CEMIP	cell migration inducing hyaluronidase (also KIAA1199 and hyaluronan binding protein (HYBID))
CEMIP2	cell migration inducing hyaluronidase 2 (also transmembrane protein 2 (TMEM2))
ECM	extracellular matrix
GAG	glycosaminoglycan
GlcNAc	N-acetyl-β-D-glucosamine
GlcUA	β-D-glucuronic acid
HA	hyaluronan (also hyaluronic acid or hyaluronate)
HAS	hyaluronan synthase
HYAL	hyaluronidase
IL-1	interleukin 1
IVD	intervertebral disc
OA	osteoarthritis
PCM	pericellular matrix
PG	proteoglycan
PRG4	proteoglycan 4 (also lubricin or superficial zone protein)
PTH	parathyroid hormone
RA	rheumatoid arthritis
RANKL	receptor activator of nuclear factor kappa-B ligand (also tumor necrosis factor ligand superfamily member 11, osteoprotegerin ligand, or osteoclast differentiation factor)
RHAMM	receptor for hyaluronan-mediated motility (also hyaluronan-mediated motility receptor)
TGFβ	transforming growth factor beta
TNFα	tumor necrosis factor alpha

TSG-6	tumor necrosis factor-inducible gene 6 protein (also tumor necrosis factor-stimulated gene 6 protein)