Different molecular weights of hyaluronan research in knee osteoarthritis: A state-of-the-art review

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https://doi.org/10.1016/j.matbio.2023.02.006

Abstract

Osteoarthritis (OA), the most common form of arthritis, is characterized by progressive cartilage destruction, concomitant adaptive osteogenesis, and loss of joint function. The progression of OA with aging is associated with a decrease in native hyaluronan (HA, hyaluronate or hyaluronic acid) with a high molecular weight (HMW) in synovial fluid and a subsequent increase in lower MW HA and fragments. As HMW HA possesses numerous biochemical and biological properties, we review new molecular insights into the potential of HA to modify OA processes. Different MWs in the formulation of products appear to have varying effects on knee OA (KOA) pain relief, improved function, and postponing surgery. In addition to the safety profile, more evidence indicates that intraarticular (IA) HA administration may be an effective option to treat KOA, with a particular emphasis on the use of HA with fewer injections of higher MW, including potential applications of HA of very HMW. We also analyzed published systemic reviews and meta-analyses of IA HA in treating KOA in order to discuss their conclusions and consensus statements. According to its MW, HA may offer a simple way to refine therapeutic information in selective KOA.

Introduction

Osteoarthritis (OA), the most common joint disorder in the world, has been termed ‘wear and tear’ osteoarthrosis because it damages the articular cartilage and underlying bone to reach the end stage of burnout [1,2]. In the 21st century, increasing evidence indicates that the inflammation process facilitates an active role in pain and the vicious cycle associated with the pathogenesis of OA by inducing a variety of cytokines to deteriorate hyaluronan (HA, hyaluronate, or hyaluronic acid) and hyaline cartilage in synovial joints [3]. OA affects the entire joint, presenting with focal cartilage degradation and loss, subchondral bone deterioration, remodeling with newly formed bone formation, synovial hypertrophy, capsule thickening, and alterations of surrounding tissues [2]. The complex interacts with genetic, metabolic, biochemical, and biomechanical factors to contribute to chronic degenerative processes [1,2]. Therefore, non-uniform narrowing of the joint space, subchondral sclerosis and cysts, marginal osteophytes, loose bodies, and joint subluxation are considered radiographic characteristics of the definition of OA [1] (Fig. 1). Prompt correction of the imbalance of degenerative and reparative changes appears to reverse the progress of OA radiographically [4].

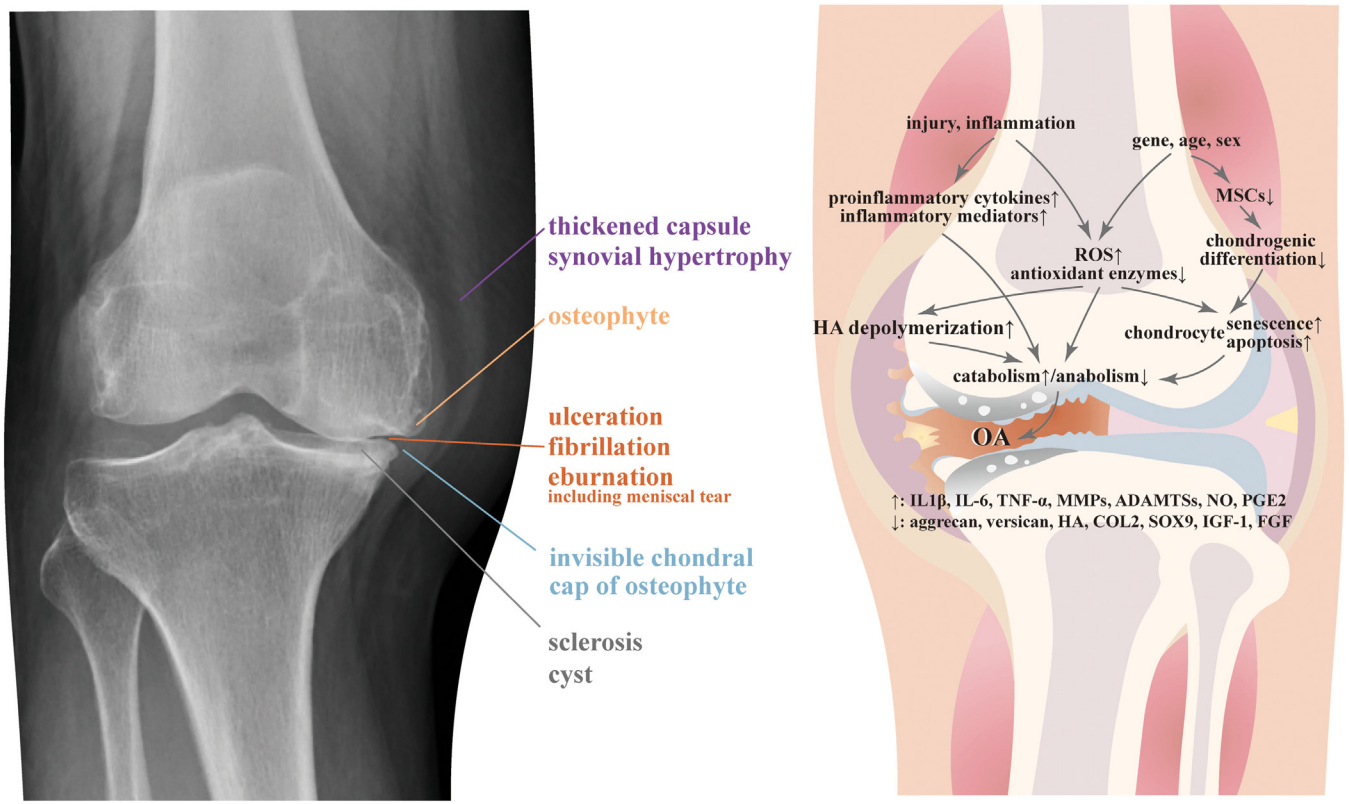

Figure 1. A radiogram of the right osteoarthritic knee and a diagram of the left osteoarthritic knee. Cartilage degradation, meniscal tear, and synovial inflammation result in the vicious cycle of OA through several pathways and mechanisms, contributing to progressive cartilage loss, pain, effusions, swelling of soft tissues, and joint dysfunction. ADAMT: a disintegrin and metalloproteinase with thrombospondin motifs; COL2: collagen type II; FGF: fibroblast-derived growth factor; HA: hyaluronan; IGF: insulin-like growth factor; IL: interleukin; MMP: matrix metalloproteinase; MSC: mesenchymal stromal/stem cell; NO: nitric oxide; OA: osteoarthritis; PGE2: prostaglandin E2; ROS: reactive oxygen species; SOX9: sex-determining region Y (SRY)-box 9; TNF: tumor necrosis factor.

OA is a debilitating multifactorial joint disease in the elderly and its etiology is not fully understood. Several risk factors accumulate to contribute to OA, including genetic, constitutional (age, sex, and obesity), biochemical (inflammation) and biomechanical predispositions (injury, occupational or recreational joint trauma, muscle weakness, malalignment, and abnormal joint shape) [2,5]. Clinically, OA is classified into those in whom the cause is unknown (primary, *de novo*) or known (secondary, for example, infection and trauma) [6]. Knee OA (KOA) causes physical disability with chronic pain, loss of function and income, and deterioration in quality of life (QOL). Substantial economic consumption and the utilization of costs for society and the health care system for KOA are responsible for approximately 85% of the burden of OA [2].

As hyaline cartilage of KOA progressively deteriorates and erodes, the underlying bony surfaces exaggerate contact pressure and enhance subchondral bone remodeling. The exposed bone underneath cartilage comes into contact with rarefaction of the bone below eburnation represented by bone 'bruise' and 'cyst' and the formation of new bone in the subchondral area and margin [1]. In femorotibial OA, an imbalanced catabolic and anabolic equilibrium at the defect site contributes to a vicious cycle of failed repair and subsequent fibrillation and disruption of the meniscus at the high-contact site, especially the inner rim of the avascular meniscus, and secondary synovial inflammation [7–9]. The decrease in molecular weight (MWs) of HA is closely associated with exacerbated surface wear, inflammation, and the risk of rapid progression of OA [10]. The development of novel treatments that target the vicious cycle caused by a torn meniscus and damaged cartilage through disease-modifying therapies is attractive. In addition to synovial inflammation, it is imperative to take into account the torn meniscus in KOA.

In addition to lacking healing capacity, the inner two-thirds of the meniscus, like articular cartilage, is aneural tissue. It is not painful when injured unless the increased mechanical pressure is transmitted to the outer third of the meniscal body and the perimeniscal tissue [11]. Without pain, the damaged inner meniscus and cartilage could further promote OA processes such as loss of sensation in neurotrophic arthropathy (Charcot joint), resulting in accelerated, non-uniform destruction of the joint space and eventual subluxation. Like chondral degradation, torn meniscal fragments released into the joint cavity then trigger secondary synovial inflammation to exaggerate the development of OA [1]. Thus, it is beneficial to develop novel treatments that target the vicious cycle caused by the injured meniscus and cartilage through disease-modifying therapeutic interventions after a better understanding of OA [7,9].

HA MWs in healthy tissues are greater than 1000 kDa, approximately 6000–7000 kDa, and the concentration is 2–4 mg/mL in the synovial fluid (SF) of a young normal knee [12]. Upon depolymerization of HA, approximately 1900 kDa and 1 mg/mL are in the SF of an older patient with KOA [13]. A shift in the MW distribution of HA in SF toward lower MW is closely associated with exacerbated surface wear and the risk of rapid progression of OA [10,14]. Native HA hampers inflammatory cell activity and the free movement of inflammatory mediators and lytic enzymes and also increases chondrocyte metabolism through molecular size [15,16]. It is believed that HA fragments are associated with pathological states to stimulate the immune system. An increase in the severity of OA, correlated with the decline in the concentration and MW of HA in SF, decreases the viscoelasticity of SF and increases susceptibility to cartilage degradation, resulting in surface wear, cartilage loss, inflammation, and pain [10,17]. Intraarticular (IA) HA products with different MW through the development of engineered tissue, sources, and biomaterials have demonstrated divergent therapeutic efficacy for the clinical treatment of OA, especially with fewer injections of higher MW HA.

Molecular physiology of the joint

Homeostasis of cartilage

Articular cartilage, ranging from 1 to 3 mm thick, has fewer chondrocytes, which are widely but rarely spread among the extracellular matrix (ECM) without containing blood vessels (avascular), lymphatic ducts (alymphatic), or nerves (aneural) [18]. Chondrocytes produce the main structural components of ECM molecules, which mainly include collagen type II (COL2) and proteoglycans (e.g., aggrecan and versican), and maintain a firm and solid balance between synthesis and degradation to maintain cell survival [1]. A major source of nutrients and oxygen for avascular cartilage is delivered by the double diffusion system, where materials cross the SF barrier into cartilage and diffuse in the ECM to reach chondrocytes at different depths of the chondral tissue [17]. Therefore, articular cartilage has limited self-repair ability for functions including joint support and protection, lubrication and conformation, and load bearing and shock absorption [18]. Similarly, only 25–33% of the peripheral area of the meniscus remains vascular by the capillaries of the joint capsule and the synovial membrane, and vascularity declines with age, resulting in the inability of the inner two-thirds without the vascular portion to heal after damage [11].

The highly organized structure of cartilage can be divided into four zones: the superficial (tangential) zone, the middle (transitional) zone, the deep (radial) zone, and the calcified zone, and then the chondrocytes and ECM vary their distribution in each zone [5,19]. ECM constitutes 90% of the dry weight of cartilage and consists of collagenous fibers, proteoglycans, HA, and sometimes elastin [20]. Among them, collagen fibers (75% of the dry weight) are the highest in the superficial zone, decreasing by 20% in the middle and deep zones, and proteoglycans (20−30% of the dry weight) are the lowest in the superficial zone, increasing by up to 50% in the middle and deep zones. Proteoglycans comprise a core protein and one or more covalently attached glycosaminoglycan (GAG) chains, which are the main component of the ECM of virtually all mammalian tissues with different compositions of monosaccharides and glycoside binding positions [21].

HA belongs to the GAG family, which consists of non-sulfated HA and five sulfated members (chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin, and heparan sulfate). HA is present in the intracellular cytoplasm and nucleus and is involved in the cell cycle, stress conditions, and specific pathological conditions [22]. Cell stress at physiological levels of glucose initiates the synthesis of a monocyte-adhesive HA ECM, whereas dividing cells in the hyperglycemic medium initiate the HA synthesis in intracellular compartments of the cytoplasm and nucleus to induce endoplasmic reticulum stress and autophagy [23]. Unlike other GAGs, HA does not covalently link to the aggrecan core protein, but it non-covalently binds to the G1 domain of the N-terminal end of the core protein to intertwine between collagen fibrils [17]. Through the G1 domain, HA offers a backbone to link the side chains of aggrecan to form a hydrophilic aggregating proteoglycan [24]. As part of a large complex holding water in the matrix, HA allows cartilage to resist mechanical forces

In cartilage, there is a balance affected by age and regulated by several factors, including growth factors, cytokines, aggrecanases (a disintegrin and metalloproteinase with thrombospondin motifs, ADAMTSs), and matrix metalloproteinases (MMPs), which are produced by chondrocytes and type B fibroblast-like synoviocytes [1,25]. The degradation products of cartilage macromolecules and neighboring chondrocytes stimulate cellular responses to establish a positive feedback loop that involves the production of cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α. Proinflammatory cytokines work in an autocrine and paracrine way to create their inflammatory milieu and exaggerate a vicious cycle if the repair mechanism is disrupted [1].

Roles of the synovial membrane

The synovium comprises the inner layer or intima, which consists of one-third of the synovial membrane type A phagocytic cells and the most prevalent type B secretory cells, and a deep layer or subintima [17]. Type A macrophage-like synoviocytes, derived from hematopoietic monocytes, are responsible for removing degraded cartilage debris. Type B cells, known as fibroblast-like synoviocytes, secrete HA, lubricin, and other components into SF. The subintima, up to 5 mm thick, is an intricate network of fenestrated capillaries and lymphatics, which provide nutrients for avascular cartilage and drain SF waste [26]. Together with intima cells, the subintima seals SF in the joint cavity from the surrounding tissue and produces hydraulic resistance to allow an ultrafiltrate of plasma to penetrate its capillary wall and the synovium into the joint cavity and *vice versa* (waste of cartilage from SF into the capillaries and lymphatic ducts in the subintima).

Equilibrium of synovial fluid

SF contains three constituents, HA, lubricin (a surface-active mucinous glycoprotein), and phospholipids in physiological concentrations, to synergistically adjust the ability of the boundary lubricant along with effective boundary friction and shock absorption in cartilage [27]. They are physiologically responsible for the cytoprotective effects and tissue homeostasis of articular cartilage, synovia, capsules, and ligaments from mechanical damage. HA and lubricin are the main determinants of viscosity and lubrication in SF, which can be distinguished from plasma by the presence of these two molecules. In addition to lubrication, SF functions primarily as a viscous fluid rather than an elastic fluid throughout the range of joint movement [26]. Additionally, SF provides the main nutritional source for avascular cartilage, including the inner rim of the meniscus.

Lubricin is a superficial zone protein that coats the cartilage surface and plays a crucial role in cartilage integrity. Although HA is viscous, lubricin lubricates the joint surface, facilitating efficient glide [28]. Furthermore, lubricin acts as a physiological suppressor of cartilage wear, cell and protein adhesion, synovial cell proliferation, and chondrocyte apoptosis. Thus, injury, inflammation, and genetically mediated reduction in lubricin levels in both cartilage and synovium result in accumulated chondral damage and meniscal tear and the development of KOA [26].

Hyaluronic acid

Brief history of hyaluronic acid

In 1934, Karl Meyer and John Palmer first isolated HA from bovine eye vitreous humor and named it hyaloid (the Greek word hyalo means 'glass' for vitreous) and uronic acid (one of the sugar molecules) [29]. HA was also isolated from human umbilical cords, rooster comb, and streptococci in the 1930s and 1950s [30,31]. Since the 1940s, the physicochemical properties of HA have been widely demonstrated [32]. HA was further studied for biological roles after Karl Meyer and Bernard Weissmann illustrated its chemical structure in 1954 [33,34]. Viscosupplementation with HA in OA therapy was first proposed by Endre A. Balazs in 1971 [35]. Researchers isolated and purified nearly pure HA before the 1970s and the first pharmaceutical HA was extracted and purified from the polymer of rooster combs and human umbilical cords in 1979 [36].

The term 'hyaluronan' was introduced to comply with the polysaccharide nomenclature as an alternative to 'hyaluronic acid' in 1986 [35]. In recent decades, effective and safe treatments using HA have been studied in the knee and other joints [37]. The hypothesis that IA injection of HA in OA could restore the rheological properties of SF and promote the synthesis of higher MW and potentially more functional HA to offer pain alleviation, mobility, and articular function has been postulated [38]. To avoid potential side effects related to avian allergies, bacterial fermentation techniques were developed and progressively optimized to produce HA with controlled size and polydispersity [36]. However, multiple injections of most HA products were required to achieve efficacy in the treatment of OA due to their short residence (that is, faster resorption) in the synovial joints due to lower MW [17]. The purpose of this review is to discuss the pros and cons of HA with higher and lower MW.

Biosynthesis of hyaluronic acid

Non-protein HA, $(C_{14}H_{21}NO_{11})_n$ is a linear GAG (heteropolysaccharide, mucopolysaccharide), which has the same structure consisting of repeated disaccharides of glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc), linked by the β-1,3 glycosidic bond [22,36] (Fig. 2A). Unlike other relatively small GAGs with MW $<$ 50 kilodaltons (kDa), usually 15−20 kDa, the disaccharide unit repeats thousands of times by β-1,4 glycosidic linkage to form an unbranched and very long linear anionic biopolymer with a semi-flexible structure to adopt an expanded random wormlike coil [22], which can reach more than millions [17,22]. As it is self-standing, i.e. without an association with a core protein, HA is distinctively synthesized as a free polymer by three HA synthases (HASs) on the inner surface of the cellular membrane and translocated into the extracellular space along with an extension of the repetitive sequence. The synthetic process is unique compared to that of other GAGs that are completely synthesized by Golgi enzymes in the Golgi apparatus in the intracellular space [22].

Mesenchymal stromal/stem cells (MSCs) are considered to be the main source of HA [39], whereas HA in SF can be produced by several types of cells, including chondrocytes and type B fibroblast-like synoviocytes [17]. HAS1 (human and mouse), 2 (human and mouse), and 3 (mouse) isozymes are a family of transmembrane glycosyltransferases that synthesize HA with different MWs and their genes are located at 19q13.41, 8q24.12, and 16q22.1, respectively, on three different chromosomes with 50−71% identity [3]. HAS1 and HAS2 generally synthesize larger HA (MW $\geq$ 120−2000 kDa), and HAS3 synthesizes smaller molecules (MW 120−1000 kDa) [40]. Furthermore, the main population of HA tends to be longer molecules (MW $>$ 2000 kDa), which are generated by HAS2 compared to HAS1 [41], and HAS1 produces a wider range of MW polymers, including the lowest MW (120 kDa) molecule.

Physical functions of hyaluronic acid

Although HAS2, the most abundant isoform expressed in chondrocytes, produces the highest MW HA implicated in maintaining the cartilage matrix, HAS1 synthesizes the HA coat to participate in various biological functions in the pericellular microenvironment [40,41] (Fig. 2B). While HA produced in the synovial joint fills the intercellular space of cartilage, which serves as a cellular coat and a water reservoir, most of them ultimately flow into SF [35]. HAS1 and HAS2 in the synovium of patients with OA are lower than in healthy controls, so synovial HA production is decreased, including higher MW HA [3,36].

In the meshwork, HA acts as a semi-permeable barrier that regulates metabolic exchanges between cartilage and SF to support tissue structure and regulate cell signaling according to its polymer length [42]. After the formation of a rigid helix, the enormous size and hydrophilicity of the HA macromolecule attract a large amount of water to organize the broad domains of the tertiary polymer structure [43]. By facilitating mutual macromolecular crowding and binding interactions, HA viscoelastic rigidity exhibits lubrication, water homeostasis, filtering, and exclusion in tissues, as well as adhesion, motility, and organization in cells [22,44].

HA in SF forms a left-oriented individual or twin spiral, a double helix structure, and a superhelix

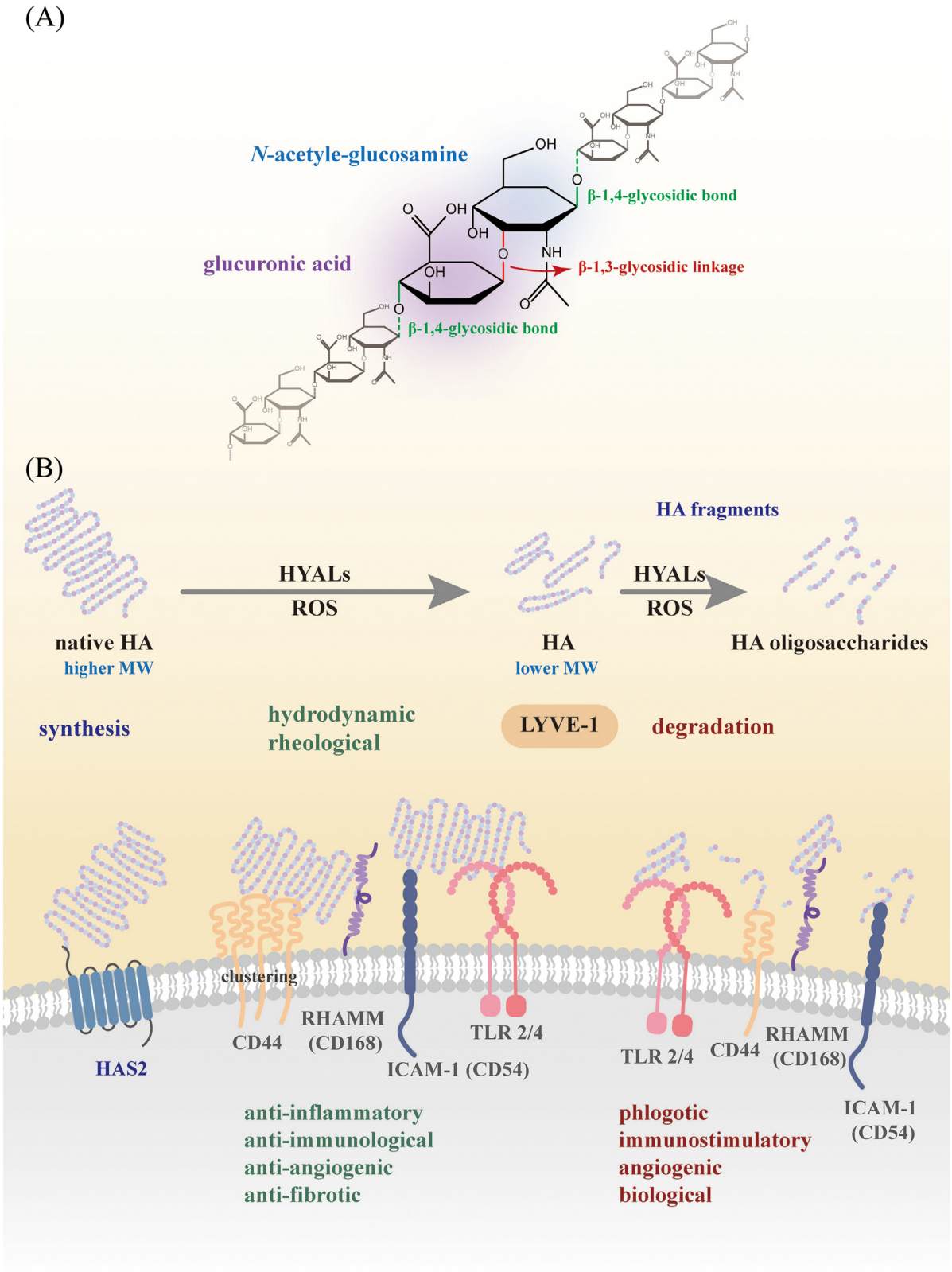

Figure 2. A summary of the chemical structures of HA and its synthesis, functions, degradation, and bioactivity. (A) HA consists of repeated disaccharides of glucuronic acid (GlcUA) and N-acetyl-glucosamine (GlcNAc), linked by the β-1,3 glycosidic bond, and the disaccharide unit repeats thousands of times by β-1,4 glycosidic linkage to form an

organization to mold semi-rigid coiled chains, helix bands, and even helical rings [45]. As HA concentrations increase, intramolecular networks are created, while three-dimensional (3D) web structures are organized, and free polysaccharides fold into a roll with increasing biopolymer concentration [12]. While intermolecular hydrogen bonds influence the structure of HA, the secondary structure of HA looks like flat bands that are transformed into a helix or twisted into a sheet [43,46].

Bioactivity of different molecular weight hyaluronic acids

Native HA in cartilage is firmly trapped in the collagenous meshwork even at a very low concentration of 1 μg/mL to capture a large amount of water, binding up to 1000× weight to form a voluminous mesh [15]. HA fragments comprise lower MWs (< ~500−700 kDa) and much smaller HA oligosaccharides (8−30 dimers), and they are synthesized *de novo* or generated by degradation of hyaluronidase (HYAL) or oxidative hydrolysis of native HA under pathological conditions, including repair, inflammation, and OA [15,16].

The structural, hydrodynamic, rheological, physicochemical, bioactive, and degradable characteristics of HA depend greatly on the MW: (1) MW of 0.4−4.0 kDa serves as a stimulator of heat shock proteins (Hsps) and non-apoptotic function; (2) MW of 6−20 kDa has phlogotic, immunostimulatory, and angiogenic effects; (3) MW of 20−200 kDa participates in biological processes such as wound healing; (4) MW > 500 kDa works as a space filler and a natural immunological depressant with anti-angiogenic behavior [47]. Higher MW HA is more cohesive than lower MW polymers, but lower MW HA cannot have the same chain entwinement value as higher MW macromolecules [15].

In cartilage, covalent and non-covalent HA-protein assemblies are indispensable for maintaining an expanded matrix surrounding the cell [15]. In SF, a wider HA network is extended with increasing concentrations and MWs, and thus, HA solutions show gradually increasing viscoelasticity. Under dynamic and static conditions, HA attached to proteoglycan aggrecan molecules is completely responsible for the rheological properties of the cartilage matrix and SF to minimize axial forces and friction on the articular surface [35]. Native HA provides shock absorption during high-shear rapid movement, such as when running, and lubrication during low-shear slow movements, such as walking [15]. Therefore, HA has beneficial mechanical and biological properties in the cartilage ECM and SF of healthy and osteoarthritic joints [48].

Hyaluronic acid receptors

Different forms of HA exert a variety of anti-arthritic effects through pain relief and multiple possible underlying disease-modifying mechanisms that involve receptors, enzymes, and other metabolic pathways, including stimulation of chondrocyte growth and metabolism, as well as inhibition of chondrodegradation, chondrocyte apoptosis, and destructive inflammation [49,50]. One of the mechanisms underlying the polymer length-dependent transduction of the HA signal is the differential clustering of HA receptors triggered by their multivalent interaction with HA [15]. Furthermore, internalization of HA mediated by receptor cluster of differentiation-44 (CD-44) is mediated by pinocytosis-, clathrin-, and caveolin-independent pathways in chondrocytes, and the intracellular CD44 domain is responsible for endocytosis of HA [22]. In cartilage, CD44-dependent signaling affects both the chondrocyte survival pathways and the chondroptotic pathways [15].

HA cell surface receptors include (1) CD44, which is a transmembrane glycoprotein that mediates HA endocytosis, (2) receptor for HA-mediated motility (RHAMM) or CD168, which cooperates with CD44 in HA endocytosis and signaling, (3) lymphatic vessel endothelial HA receptor (LYVE)-1, also known as cell surface retention sequence binding protein (CRSBP)1, which is the main HA receptor in the lymphatic vessel endothelium, and (4) HA receptor for endocytosis (HARE), which mediates type I single-pass membrane protein-mediated HA internalization, and others such as intercellular adhesion molecule (ICAM)-1 (CD54) [15,22].

The interaction of CD44 and HA is strongly influenced by cell-specific factors, cell types, the state of CD44 activation, and the size of the HA ligand. Higher MW HA carries repeated disaccharides, which accumulate up to a few thousand CD44 binding sites and interact simultaneously with many CD44 molecules on the cell surface [15]. Through receptor clustering and engagement, endocytosis, intra- and extracellular signaling, and interactions with HA ligands such as heavy chain domains of inter-α-inhibitor (IαI) family molecules and TNF-α-stimulated gene (TSG)-6, different MW HA

unbranched and very long linear anionic biopolymer. (B) The biosynthesis, function, degradation, and physicochemical bioactivity of different MW HAs depend on the cell type. CD: cluster of differentiation; HA: hyaluronan; HAS; hyaluronan synthase; HYAL: hyaluronidase; ICAM: intercellular adhesion molecule; LYVE: lymphatic vessel endothelial hyaluronan receptor; MW: molecular weight; RHAMM: receptor for hyaluronan-mediated motility; ROS: reactive oxygen species; TLR: Toll-like receptor.

potentially displays a cornucopia of biologic properties and their therapeutic diversity [15].

As a result of stimulation with a high MW (HMW) HA, CD44 clustering is a typical response characteristic [51]. Although long chains of HA contain multiple or repetitive sites for selective promotion of CD44 clustering, smaller MW HA has only one to two CD44 binding sites for disrupting them. CD44 binding of higher MW HA inhibits cell proliferation, whereas binding of lower MW HA exhibits a stimulating effect [52]. Therefore, HA with higher MWs exerts anti-inflammatory, anti-angiogenic, and anti-fibrotic properties, including repressing cell proliferation, migration, and sprout formation, but HA with lower MWs promotes endothelial cell proliferation and motility, producing proinflammatory and pro-angiogenic reactions via CD44 [53]. Even when HA is present in the cell, either uptake from the ECM or synthesized by cytoplasmic HASs in hyperglycemic dividing cells, intracellular HA exerts diverse functions under specific pathological conditions [22].

Depolymerization of hyaluronic acid

HA is depolymerized by two different mechanisms in the human body [50]. One is specific and determined by enzymatic hydrolysis of HYALs, including six members of the HYAL family, HYAL1, 2, 3, and 4, HYALP1, and PH-20/sperm adhesion molecule 1 (SPAM1) [47]. The other depolymerization is non-specific and is mediated by radical scission via reactive oxygen species (ROS) and free radicals due to oxidative damage. Homologous isozymes HYAL1, 2, and 3 of the β-endoglycosidase family are clustered on human chromosome 3p21.3, and they cleave HA into small fragments by destroying internal β-1,4 bounds. The genes HYAL4, HYALP1, and PH-20/SPAM1 are located on chromosome 7p31.3 [54].

In an aqueous solution, its rheological properties of HA are influenced by ionic strength, temperature, and pH, for example, a reversible reduction of the viscosity in alkaline HA solutions [36]. HA depolymerization in alkaline solutions is more powerful than in acidic conditions because of the disruption of H bonds. The MW of HA in cartilage and SF is reduced by HYAL hydrolyzing the hexosaminidic β-1,4 glycosidic linkages [55]. Higher MW HA interacts with their main receptor CD44 on the cell surface where HYAL2 is located, and HYAL2 rapidly cleaves HA to approximately 50 disaccharides (about 20 kDa fragments) to slow its activity [47,50]. HYAL2 activity is recognized to be under the control of pH decreases, such as the acidic pH of the persistent inflammatory milieu. HYAL1, a rapid acidic lysosomal enzyme, cleaves all sizes of the HA chain, including small fragments of the tetrasaccharide after HYAL1 cleavage [22]. The two enzymes operate independently and coordinately and are associated with CD44 extra- and intracellularly.

Bioavailability of hyaluronic acid

After oral intake of HA in rats and dogs, intestinal absorption and transport of HA into and out of the synovial space, as well as tissue distribution, are suggested to be by the lymphatics before the blood [56] (Fig. 3A). Intraperitoneal (i.p.) administration of HA into the bloodstream from the interstitium is through end lymphatic openings and uptake of visceral lymphatic vessels in dialysis rats. After intravenous (i.v.) injection of HA in healthy adults and rabbits, HA shows 2.5−5.5 min of plasma clearance half-life [57]. Therefore, exogenous HA by IA injection is more direct and effective than oral, i.p., or i.v. administration. After IA injection, HA diffuses mainly into the surrounding tissue and dissipates from the joint through lymphatics and blood vessels.

In adult humans, HA is estimated to be approximately 11−17 g (about 15 g in an average 70 kg human body) [17]. Although HA has a high rate of turnover in the circulation, approximately 5 g per day in humans, and inert tissue such as cartilage, for approximately 1−3 weeks, HA turnover in SF is fast, with a $t^1/_2$ of approximately 12 h [47]. The liver and kidney systems are responsible for the clearance of HA from the bloodstream, whereas different MWs of HA determine the biodistribution pathways in circulation [57,58]. Most of the elimination of daily turnover HA, at least 150 mg, is catabolized by lymphatic removal and then degraded in lymph nodes and liver endothelial cells [57]. Small MW fractions with a maximum MW limit for renal excretion of 25 kDa and the main fraction in the range of 4-12 kDa are found and eliminated in human urine [57,59].

In the knee, HYALs and ROS locally degrade approximately 30% of HA into small fragments [50,60]. The remaining 70% of HA and fragments are absorbed by macrophagic type A synovial cells, or sieved from fluid in the joint cavity and enter the systemic circulation. After leaving the joint, approximately 90% of HA is transported primarily to the lymph nodes, which are the main site of clearance, to be removed and metabolized by LYVE-1 [3,50]. The remainder is transported into the bloodstream and the endothelial cells in the liver, kidney, and spleen subsequently catabolize most of them. While 90% of HA of the remainder in the circulation is taken up in the liver and a small part in the spleen, the kidney extracts the remaining 10% of HA from the circulation, only 1−2% of the total amount of HA [48,50]. Therefore, the serum HA levels of osteoarthritic patients are significantly higher than those of their human counterparts and correlate with the severity of radiographic KOA [10]. The cascade of HA depolymerization in OA SF is associated with increased friction on the cartilage surface; thus the

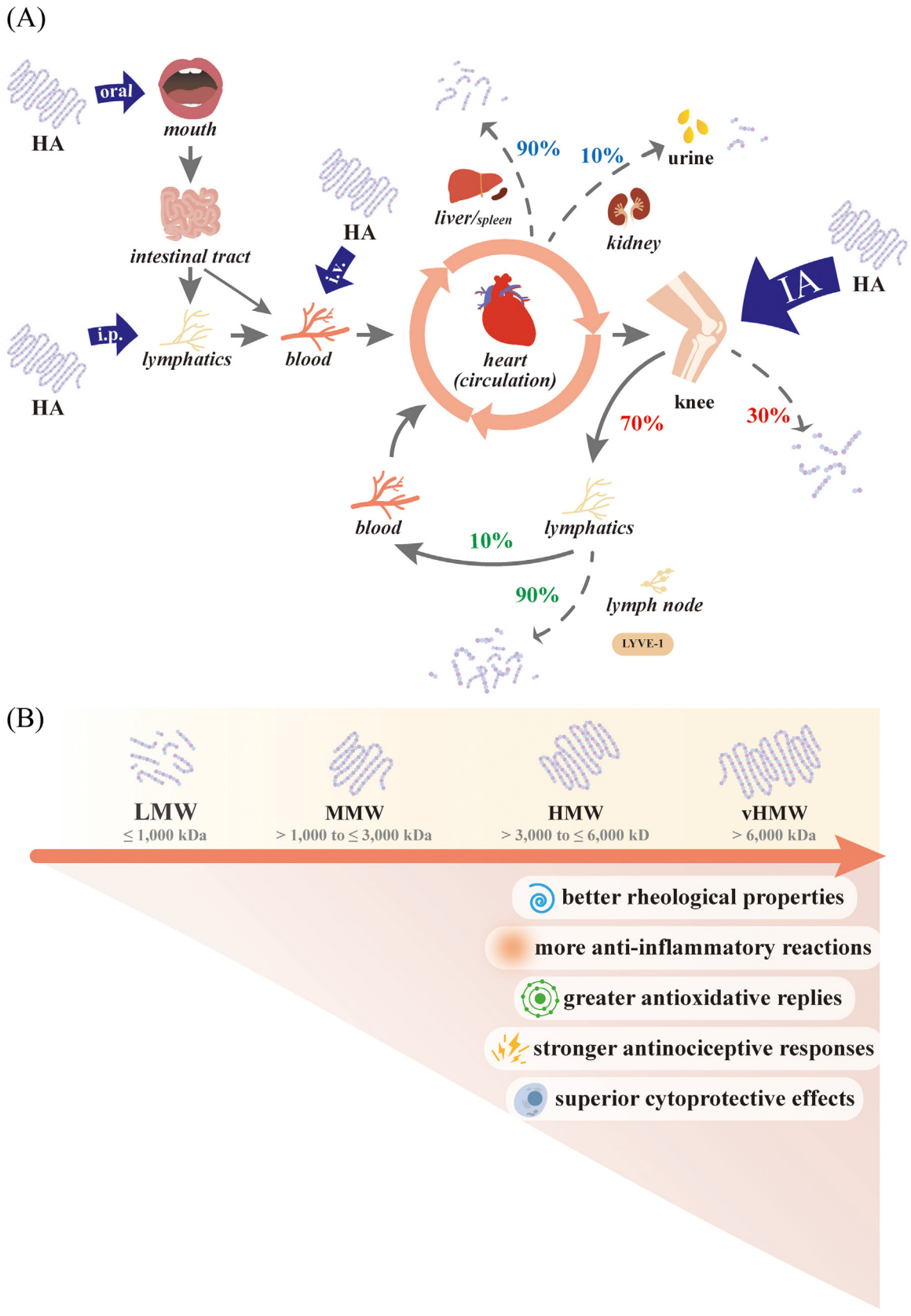

Figure 3. Diagram of the absorption, metabolism, circulation, and excretion of HA in the body, and their difference in the rheological properties, anti-inflammatory responses, antioxidants scavenging, analgesic

changes provide the relevance of higher MW HA viscosupplementation [3,61].

Pathogenesis of osteoarthritis

Vicious breakdown of cartilage

In the regulation of chondral homeostasis, chondrocytes function as mechanosensor to influence ECM composition, organization, and ultimately the mechanical resilience of the cartilage [62]. Chondrocytes sense changes in interstitial fluid, osmotic stress, water pressure, and flow potential through mechanoreceptors, including the integrin receptor, the primary cilium, and the ion channel on the cell membrane, to transmit extracellular physical stimulation and cytoskeletal complex signals for intracellular communication. During aging, chondrosenescence, and metabolic alterations, chondrocytes lose the ability to maintain cartilage homeostasis. This result in the decline in mitotic and synthetic activity to respond to anabolic growth factors and synthesize cartilage-specific proteoglycan core proteins, as well as to increase the demand for the biosynthesis of proinflammatory and degradative proteins [63].

When cartilage damage is progressive, chondrocytes undergo a metabolic change in the regulation of inflammatory responses and a phenotype change characterized by an increase in the production of cartilage-degrading enzymes, for example, MMPs and aggrecanases, and decreased expression of the sex-determining region Y (SRY)-box 9 (SOX9) [1,63]. Catabolic MMPs and aggrecanase-1 and 2 (ADAMTS4 and 5), which are members of the ADAMTS family, mediate the degradation of COL2 and aggrecan, respectively [1]. In mouse and human osteoarthritic cartilage and synovium, the frizzled gene increases expression and activates the Wnt/β-catenin pathway to regulate chondrocytes and macrophages and further enhance matrix destruction through MMPs and aggrecanases [64]. Chondral impairment with loss of integrity and thickness has been ongoing as a consequence of the decrease in proteoglycan, COL2, and HA [1].

Eroded cartilage in mice, sheep, and human OA increases mechanical loading and focal chondrocyte sclerostin [a vigorous inhibitor of Wnt signaling by binding to low-density lipoprotein receptor-related protein (LRP)5 and 6], but decreases the expression of subchondral osteocyte sclerostin, contributing to the localized bone formation with a resultant increase in focal bone remodeling [65]. Despite the opposing effects of osteocyte sclerostin on the promotion of subchondral sclerosis and inhibiting chondral degradation, the net rate of chondral ECM synthesis reduces, and its degradation increases [66]. These effects result in a decrease in load-bearing capacity and an increase in chondrocyte apoptosis leading to further hypocellularity in the hyaline and fibrous cartilage. In such situations, the prompt delivery of nutrients and oxygen to the decreased chondrocytes through the double diffusion systems of the SF and cartilage barriers becomes critical. Chondrocytes derive adenosine triphosphate (ATP) from glycolysis and undergo profound ATP depletion with increased production of nitric oxide (NO) during periods of low oxygen availability [63]. Furthermore, decreased mitochondrial respiration and excessive ROS production in chondrocytes result in oxidative damage.

Characteristics of meniscal tear

In the femorotibial joint, the meniscus offers shock absorption, aids in load transmission and lubrication, joint stability, limitation of extreme flexion and extension, prevention of synovial impingement, and chondral nutrition supply. Meniscal tear leads to increased loading pressure and decreased distribution and nutrition of SF, resulting in cartilage degradation and ECM breakdown, as well as *vice versa* since chondral injury also contributes to meniscal degeneration at the interposing site.

KOA shows similar pathological changes in degraded cartilage in the degenerative and torn meniscus, including cell clusters and death, calcification, ECM disruption, and surface fibrillation [8]. Accelerated mechanical status and elevated expression of messenger ribonucleic acid (mRNA) of gelatinases (MMP-2 and 9) and membrane-type 1 MMP caused by uneven contact surface and impingement

pharmacotherapy, and cytoprotective effects in osteoarthritic knees. (A) IA HA is more direct and efficacious than oral, i.p., or i.v. administration, and approximately 30% of HA are locally degraded into small fragments by HYALs and ROS. The remaining 70% of HAs and fragments dissipate out of the joint and diffuse out into the surrounding tissue via lymphatics, bloodstream, and circulating throughout the body. After leaving the joint, approximately 90% of HA is degraded in the lymph nodes and the remaining 10% of HA is mainly taken up in the liver (90%) and the remainder (10%) is extracted from the kidneys. (B) According to MW, current IA HA products can be defined into four groups: LMW ($\leq$ 1000 kDa), MMW ($>$ 1000 to $\leq$ 3000 kDa), HMW ($>$ 3000 to $\leq$ 6000 kDa), and vHMW ($>$ 6000 kDa). HA: hyaluronan; HMW: high molecular weight; IA: intraarticular; i.p.: intraperitoneal; i.v.: intravenous; kDa: kilodaltons, LMW: low molecular weight; LYVE: lymphatic vessel endothelial hyaluronan receptor; MMW: medium molecular weight; vHMW: very high molecular weight.

of the torn meniscus in human OA gradually destroy the local meniscal and chondral matrix [7]. In a sheep meniscectomy-induced OA model, an alteration of Wnt/β-catenin signaling, which is needed to maintain normal cartilage, is also observed [65]. Pathologically, these physical and chemical actions lead to softening, weakening, thinning, and eventually destruction of meniscal fibrocartilage and articular hyaline cartilage.

Secondary synovial inflammation

To clean the worn particles and debris, chondral erosion and meniscal tear participate in hyperplasia, angiogenesis, and a secondary inflammatory reaction of the synovium [1,67]. Type B synoviocytes are stimulated to carry more proteinases, nutrients, and immune cells into SF to destroy damaged fragments and repair damaged tissue. However, the highly disruptive effects of proinflammatory cytokines (e.g., powerful catabolic factors IL-1β, IL-6, and TNF-α), inflammatory mediators, ROS, and MMPs act directly on normal chondrocytes to disarrange cartilage homeostasis and collectively drive the OA process [7]. In summary, the breakdown of ECM components outweighs the synthesis of new ECM through up-regulation of catabolic and inflammatory responses, down-regulation of anabolic events, and hypertrophic differentiation of synovia, leading to increased cartilage deprivation [1].

With the vicious cycle continuing, excessive proinflammatory cytokines, inflammatory mediators, and ECM-degrading enzymes are produced, more ECM is degraded, more cartilage loss is evolved, inflammation is constantly cascaded, and cartilage homeostasis is eventually dismantled [1]. Advancing the inflammatory cascade further destroys cartilage and ECM by increasing the breakdown of COL2, the main component of cartilage, and by reducing the expression of anabolic pathway mediators by inhibiting the major transcription factor of COL2 and SOX9, resulting in chronic low-grade inflammation and cyclic destruction of the synovial joint [1,63].

Degradation of hyaluronic acid

Age- and injury-related OA eventually results from both imbalances in cartilage degradation and synthesis processes. In other words, the catabolic process exceeds the anabolic process. Deprivation of chondral tissue and depletion of the rheological properties of SF are characteristics of OA. HYALs and macrophage-generated ROS/reactive nitrogen species (RNS) concomitantly degrade macromolecule HA, leading to the loss of higher MW HA and a subsequent increase in the concentration of lower MW HA in SF [68]. Thus, lower MW polymers, resulting from the depolymerization of higher MW HA [10], perhaps down to a few hundred kDa are generated and accumulated. Then these fragments interact with a different set of receptors to trigger signaling cascades [15].

Together, HA concentration and size are reduced, so HA concentration and MW in SF of osteoarthritic patients are lower than those of healthy humans [69]. As a result of synovial inflammation and cartilage degradation, and therefore, the serum HA levels of osteoarthritic patients are significantly higher than those of their human counterparts. Furthermore, the serum HA level correlates with the severity of radiographic KOA [10]. As native HA depolymerizes in the SF of OA, friction on the cartilage surface increases; this provides the rationale for providing higher-MW HA viscosupplementation [70].

Medical treatment of knee osteoarthritis

As KOA is chronic and nonfatal, the disease causes a significant deterioration in QOL and impacts the socioeconomic burden and the health care system [71]. Joint pain and disability are the main reasons for patients with KOA to seek medical care, including symptom- and structure-modifying therapies [72–75], arthroscopic surgery or osteotomy for early-stage OA, and prosthesis replacement or arthrodesis for late-stage OA [4,7]. However, arthrodesis completely affects joint functions, and knee replacements are often associated with serious life-threatening complications such as thromboembolism and periprosthetic infection [76]. The high prevalence of KOA and the current lack of available disease-curing drugs have resulted in a rise in regenerative medicine efforts.

Non-pharmacological management of knee osteoarthritis

Non-pharmacological management of KOA includes patient education (e.g., weight loss), physical therapy (e.g., quadriceps strengthening and aerobic exercise), and occupational therapy. They should be tailored based on knee risk factors (obesity, adverse mechanical factors, and physical activity), general risk factors (age, comorbidity, and polypharmacy), level of symptoms (pain, intensity, and disability), signs of inflammation (e.g., effusion), and structural damage (location and degree) [77].

Pharmacological management of knee osteoarthritis

Current pharmacological strategies for KOA are primarily concerned with controlling pain, improving joint function and QOL, delaying disease progression, and, if possible, avoiding the toxic effects of therapy [78]. The use of acetaminophen, which is associated with high-dose hepatotoxicity, was once

considered a virtually risk-free analgesic and used as first-line pain therapy [77,78]. Oral nonsteroidal anti-inflammatory drugs (NSAIDs), including nonselective and selective cyclooxygenase (COX)2, can be tried if non-pharmacological therapy and acetaminophen fail to offer adequate relief of symptoms, while they are associated with gastrointestinal, cardiovascular, and renal risks [79].

Oral opioids (e.g., tramadol), commonly used for intractable pain, pose serious risks, including overdose, opioid addiction, and death, due to prolonged use, misuse, and use without medical supervision [80]. Although IA corticosteroids have been indicated for KOA with painful effusions, they cause additional chondral damage if overdosed owing to repeated and frequent injections over time; therefore, the duration of benefits often lasts only 3 months [37]. Since 1987, IA HA has been available in Japan and Italy, in Canada in 1992, in Europe in 1995, and in the United States in 1997 [81]. Additionally, evidence to demonstrate consistency and a positive response to treatment in KOA with IA platelet-rich plasma (PRP), dextrose prolotherapy, and stem cell therapy is still lacking [37,49,82–84].

Intraarticular hyaluronic acid injection for knee osteoarthritis

IA administration of HA for KOA exhibits more potential effects than oral or intravenous application based on bioavailability [56,57]. In KOA treatment paradigms, the higher degree of OA is correlated with lower concentrations and lower MWs of HA in SF, the presence of adverse events (AEs) and less efficacy of NSAIDs and corticosteroids, and a high rate of revision among younger patients as well as miserable endoprosthesis infection, leading to early consideration of HA products [61,85–88]. Both basic research and clinical trials have demonstrated the promising advantage and complementary differences in the rheological behavior of different MW HA and more evidence to support the benefit of higher MW HA for KOA [89–92].

Through inflammation, tissues and SF are remodeled, leading to an increase in HA content, but a decrease in the average MW with a broader range of sizes and fragmentation of HA, resulting in lower MWs ($<$ 250 kDa), ultimately leading to a loss of the protective HA coat [93]. The pericellular HA coat, depending on CD44–HA interactions, is usually thin and expanded by inflammatory agents and glycemic stress through activation of its producer HAS1 [41]. HA fragments further stimulate the recruitment of macrophages and other leukocytes to the injured and inflamed tissues and activate their transcription of inflammation-related genes, such as IL-1β, TNF-α, transforming growth factor (TGF)-β, and MMPs [14,41].

Briefly, exogenous HA hampers the inflammatory cascade implicated in the pathogenesis of OA by repressing proinflammatory cytokines (e.g., IL-1β, IL-6), chemokines (e.g., IL-8), proteases (e.g., MMPs, ADAMTSs), ROS, and prostaglandin E2 (PGE2) to modulate leukocyte adherence, proliferation, migration, chemotaxis, and phagocytosis through transcription factors [e.g., nuclear factor κ-light-chain enhancer of activated B cell (NF-κB), phospho(p)-p38, and p-extracellular signal-regulated kinase (ERK)] [94–97]. First-generation HA injections with relatively lower MW on average for the treatment of KOA, mostly derived from rooster combs, require multiple weekly injections for optimal efficacy. After injection, it takes approximately 26 hours to be cleared from SF through lymphatic drainage [17]. To improve the therapeutic effect and to reduce the number of injections, several emerging and conventional methods are currently being commercialized [36,98].

Molecular mechanism of exogenous high molecular weight hyaluronic acids

According to HA in SF of OA and healthy subjects, we defined the currently available IA HA products as low MW (LMW) ($\leq$ 1000 kDa), medium MW ($>$ 1000 to $\leq$ 3000 kDa), HMW ($>$ 3000 to $\leq$ 6000 kDa), and very HMW (vHMW) ($>$ 6000 kDa) for consistent comparison in this review [42] (Table 1). Interestingly, the current trend is towards higher MW preparations due to their better therapeutic effects and fewer injections to produce rheological, anti-inflammatory, antioxidative, antinociceptive, and chondroprotective responses [12] (Fig. 3B).

Better rheological properties

HA, which adopts different conformational changes in condensed, relaxed, and condensed states at various chain lengths, acts as a lubricant and shock absorber to prevent chondral degradation by reducing friction [99]. IA HA with higher MW is larger in size to enhance the viscoelastic profile and has a longer residence period in the joint space to replenish the joint cushioning effect of native HA [91,98,100–103]. Because IA HA characterizes the viscous mode and mainly resembles the viscous and elastic features of healthy SF, multiple injections are routinely required for the short residence of the lower MW of HA [35,104]. Theoretically, it is ideal to replenish the prolonged residence of HA products that are similar to native HA in terms of molecular structure and viscoelastic properties [12].

Lower MW ($<$ 200 kDa) HA affects cell behavior by binding to receptors CD44 and RHAMM, and to direct or indirect signals through Toll-like receptors (TLRs) [47,93]. The larger the HA molecule, the

higher the affinity for HA receptors [15,51]. In OA, the greater penetration of the lower MW HA through the synovial tissue facilitates its passage from SF to the lymphatics and circulation [105]. HMW (6000 kDa) Synvisc remains longer in the joint and exhibits enhanced effects of viscous properties and friction reduction to protect cartilage from mechanical degradation and delay further injection by modifying crosslinking [106].

More anti-inflammatory reactions

In inflamed tissues, the increase in HA is part of the fragmentation of ECM macromolecules into lower MW (< 120 kDa) HA and ultimately depolymerized into HA oligosaccharides by catabolizing factors, including HYALs, mechanical forces, oxidative stress, and inflammation [48]. Lower MW (< 500 KDa) forms of HA induce inflammatory responses in inflammatory cells, but not in resident macrophages, by binding to CD44 and TLRs to result in raised NF-κB, IL-1β, IL-6, IL-12, and TNF-α production [14,16]. HA fragments stimulate mRNA, protein expression, and enzyme activity of the metalloelastase and urokinase-type plasminogen activator (uPA) and PA inhibitor-1 (PAI-1) of the PA/plasmin system, as well as NO synthesis in macrophages and other cell types [16,55].

At chronic inflammatory sites, HA degradation products enhance the proliferation of chondrocytes, fibroblasts, and endothelial cells, acting as chemoattractants to accumulate inflammatory cells. HA with a lower MW of 200 kDa improves eosinophil survival mainly through partial CD44 signaling and drives activation of TGF-β production and ICAM-1 expression more than HA with a higher MW of 3000−5800 kDa [107]. The effects eventually contribute to the synthesis of ECM and airway fibrosis in patients with chronic airway inflammation. Interaction of IA HA with CD44 ameliorates TGF-β1-induced synovial neovascularization and fibrosis to maintain chondral and synovial/capsular integrity [108].

In human normal and osteoarthritic chondrocytes, HA (MW 800 kDa) demonstrates a potential structure-modifying effect by down-regulating IL-1β-stimulated MMPs, such as MMP-1, 2, 3 (stromelysin), 9, and 13 by clustering CD44 [95]. Furthermore, HA with higher MW (2700 kDa) represses more IL-1α-stimulated ADAMTS4 (aggrecanase-1) through CD44 and ICAM-1 signaling than those with lower MWs (1.2, 300, and 800 kDa) in human osteoarthritic chondrocytes [96]. In human synovial tissue, HA with 800 kDa MW suppresses IL-1β binding to its membrane-bound receptor to down-regulate MMPs (MMP-1, 3, and 13), contributing to the rescue of inflamed osteoarthritic joints from bone and cartilage destruction, but without influence on the tissue inhibitor of metalloproteinase-1 (TIMP-1) in cartilage or synovium of mild rabbit OA [97].

In addition to maintaining homeostasis and tissue integrity, HA with increasing MW adjusts the behavior of macrophages, lymphocytes, mast cells, adipocytes, and immune cells [14,109]. Higher MW (1900 kDa) HA exerts more inhibitory chemotaxis and, to a lesser extent, phagocytic functions of neutrophils than HA with lower MWs of 800 kDa and 300 kDa [110]. Conjointly, HA with higher MW provides a correlation of anti-inflammatory effects by suppressing proinflammatory mediators to reduce human synovial cell proliferation, ADAMTSs and peroxisome proliferator-activated receptor (PPAR)γ expression, PGE2 synthesis, and arachidonic acid (ARA) release [94]. Through binding to TLR-2 and 4 and ICAM-1, higher MW HA also modifies their biological capacities to down-regulate MMPs through HA−CD44 binding and inducible NO synthase (iNOS), particularly more significant effects on inhibition of PGE2 and IL-6 [111,112].

The MW-dependent anti-inflammatory effect evoked by HA mitigates biomarkers associated with cartilage degradation (e.g., chondroitin 6-sulfate, keratin sulfate) and stimulates biomarkers of ECM synthesis (e.g., C-propeptide of COL2) [113]. Higher MW (600−1200 kDa) HA has a more pronounced effect on increasing PPARγ and decreasing COX2 and MMPs than lower MW (1.22 kDa) HA oligosaccharide [94]. HA exhibits protective cartilage effects by suppressing MMPs and the MMP-3/TIMP-1 ratio and activating proteoglycan synthesis, as well as the antinociceptive effect, which implicates both symptomatic and structural modification [3]. The superior effect of cartilage protection obtained by HA is dependent, at least in part, on its MW. Synvisc, Artz (620−1170 kDa), and Hyalgan (500−730 kDa) sequentially decrease uPA, PA-1, and MMP-2 and 9 in chondral, meniscal, and synovial cultures of early OA [89]. Using HA with higher MW to treat early OA suppresses the expressions of the PA/plasmin system and MMPs to partially delay the progression of OA [7].

HA directly affects chondrocytes or synoviocytes to produce basic fibroblast-derived growth factor, insulin-like growth factor-1, and TGF-β, contributing to the prevention of cartilage degeneration and the promotion of cartilage regeneration [104]. In osteoarthritic joints, HA decreases capsular/synovial fibrosis and stimulates the synthesis of endogenous higher MW HA and proteoglycans of the ECM molecules by resident synoviocytes [108,114]. Exogenous HA with MW of 500−4000 kDa achieves a maximal effect of HA synthesis by human synoviocytes, but HA with MW < 500 kDa has no effect [115]. However, exogenous HA stimulation with MW ≥ 4700 kDa declines with increasing concentration of HA. The synthesis of HA elicited by external concentration- and MW-dependent HA is limited by optimal

Table 1. Different formulations of IA HA preparations without corticosteroids, PRP, or antioxidants.

Brand	Company/manufacturer	Composition	MW (kDa)	Source	Confirmation	Content (mg/mL)	Volume (mL)	Injection/ cycle	Total dosage (mg)	Total volume (mL)
LMW ($\leq$ 1000 kDa)										
Hyalgan®/Triluron®/Replasyn®	Fidia Farmaceutici S.p.A./Fidia Pharma USA Inc.	Sodium HA	500−730	Avian	Linear chain	10	2	3−5	60−100	6−10
HyMovis®/HYADD® 4	Fidia Farmaceutici S.p.A.	HA	500−730	Bacterial fermentation	Linear chain	8	3	2	48	6
Suplasyn®	Bioniche Life Science Inc.	Sodium HA	500−1000	Bacterial fermentation	Linear chain	10	2	3-6	60-120	6-12
Suplasyn® 1-shot	Bioniche Life Science Inc.	Sodium HA	500−1000	Bacterial fermentation	Linear chain	10	6	1	60	6
Artz®/Artzal®/ARTZDispo®/Visco-3™/ Supartz®/Supartz FX®	Seikagaku Corp./Bioventus LLC	Sodium HA	620−1170	Avian	Linear chain	10	2.5	3−5	75−125	7.5−12.5
Adant®/GenVisc850®/TriVisc®	Meiji Pharma Spain, S.A./OrthogenRx. Inc.	Sodium HA	620−1170	Bacterial fermentation	Linear chain	10	2.5	3−5	75−125	7.5−12.5
Hyajoint/Hyafelic	SciVision Biotech Inc.	Sodium HA	650−1200	Bacterial fermentation	Linear chain	10	2.5/3	3	75/90	7.5/9
Sinovial® HL	Laboratoires Genévrier	Sodium HA	1100−1400 + 80−100	Bacterial fermentation	Linear chain	16 + 16	1	2	32 + 32	2
Sinovial® (Yaral®/Intragel®)	Laboratoires Genévrier	Sodium HA	800−1200	Bacterial fermentation	Linear chain	8	2	3−5	48−80	6−10
Sinovial One®	Laboratoires Genévrier	Sodium HA	800−1200	Bacterial fermentation	Linear chain	20	2.5	1	50	2.5
MMW ($>$ 1000 to $\leq$ 3000 kDa)										
Go-On®	RottapharmIMadaus	Sodium HA	800−1500	Bacterial fermentation	Linear chain	10	2.5	3	75	7.5
Ostenil®/Hya-ject®	TRB Chemedica UK	Sodium HA	1200−1300	Bacterial fermentation	Linear chain	10	2	3	60	6
Jetknee	SciVision Biotech Inc.	Sodium HA	1500	Bacterial fermentation	Linear chain	20	2	1−3	40−120	2−6
Ostenil® Plus/Hya-ject® plus	TRB Chemedica UK	Sodium HA	1600	Bacterial fermentation	Linear chain	20	2	1	40	2
Fermathron®	Hyaltech Ltd./Zimmer Biomet	Sodium HA	1190−2030	Bacterial fermentation	Linear chain	10	2	5	100	10
Hyalubrix®	Fidia Farmaceutici S.p.A.	HA	1500−2000	Bacterial fermentation	Linear chain	15	2	3	90	6
Hyalubrix 60/HyalOne®	Fidia Farmaceutici S.p.A.	HA	1500−2000	Bacterial fermentation	Linear chain	15	4	1	60	4
GelSyn-3®	Bioventus LLC	Sodium HA	1400−2100	Bacterial fermentation	Linear chain	8.4	2	3	50.4	6
Suvenyl®/NRD101	Chugai Pharmaceutical Co., Ltd./Hoechst Marion Roussel	Sodium HA	1900	Bacterial fermentation	Linear chain	10	2.5	3−5	75−125	7.5−12.5
Orthovisc®	Anika Therapeutics, Inc./DePuy Synthes	HA	1000−2900	Bacterial fermentation	Linear chain	15	2	3−4	90−120	6−8
Monovisc®	Anika Therapeutics, Inc./DePuy Synthes	Sodium HA	1000−2900	Bacterial fermentation	Crosslinked	22	4	1	88	4
Structovial® (F60027)	Pierre Fabre Médicament	Sodium HA	2200−2700	Bacterial fermentation	Linear chain	10	2	3	60	6
Synojoynt™	Teva Pharmaceutical Industries Ltd.	Sodium HA	2500	Bacterial fermentation	Linear chain	10	2	3	60	6
BioHA/Neflexxa/Euflexxa®/Arthrease™	Savient Pharmaceuticals/Ferring Pharmaceuticals Inc.	Sodium HA	2400−3600	Bacterial fermentation	Linear chain	10	2	3−5	60-100	6-10
Hyruan Plus®	LG Life Science	Sodium HA	3000	Bacterial fermentation	Linear chain	10	2	3	60	6
HMW ($>$ 3000 to $\leq$ 6000 kDa)										
Fermathron® Plus	Hyaltech Ltd./Zimmer Biomet	Sodium HA	2300−3980	Bacterial fermentation	Linear chain	15	2	3	90	6
Fermathron® One	Hyaltech Ltd./Zimmer Biomet	Sodium HA	2300−3980	Bacterial fermentation	Linear chain	20	3	3−5	180−300	9−15
Synvisc® (hylan G-F 20)	Biomatrix Inc./Genzyme Corporation/Sanofi-Aventis	Hylan A:hylan B (80:20)	6000 (hylan A)	Avian	Crosslinked	8	2	3	48	6
Synvisc One® (hylan G-F 20)	Genzyme Corporation/Sanofi-Aventis	Hylan A:hylan B (80:20)	6000 (hylan A)	Avian	Crosslinked	8	6	1	48	6
vHMW ($>$ 6000 kDa)										
Hyruan One®	LG Life Science	Sodium HA	10,000	Bacterial fermentation	Crosslinked	20	3	1	60	3
Durolane® (NASHA)	Bioventus LLC/Smith & Nephew	Stabilized HA	100,000	Bacterial fermentation	Linear chain, ~1% Crosslinked	20	3	1	60	3
MW: N/R										
Variofill®	Adoderm GmbH	Sodium HA	N/R	Bacterial fermentation	Crosslinked	33	2	2	132	4
Hyajoint Plus®/Hyafelic Uno® (Hyvisc One)	SciVision Biotech Inc.	Sodium HA	N/R	Bacterial fermentation	Crosslinked	20	3	1	60	6
HyLink®/Gel One® (Gel-200)	Seikagaku Corporation/Zimmer Biomet	HA	N/R	Avian	Crosslinked	10	3	1	30	3
Fermathron® S	Zimmer Biomet	Sodium HA	N/R	Bacterial fermentation	Crosslinked	34.5	2	1	69	2
Jonexa™ (hyalstan SGL-80™)	Genyme Biosurgery	Hylastan: sodium HA (80:20)	N/R	Bacterial fermentation	Crosslinked	10.5	4	1	42	4

HA: hyaluronan, HMW: high molecular weight; IA: intraarticular; kDa: kilodaltons, LMW: low molecular weight; MMW: medium molecular weight; MW: molecular weight; NASHA: non-animal stabilized hyaluronan; N/R: not reported as formulation due to its crosslinks; PRP: platelet-rich plasma; vHMW: very high molecular weight.

binding to the surface of synovial fibroblasts, and this question should be further explored.

Greater antioxidative replies

Depending on its concentration and MW, the enormous macromolecular size of HA fills to cushion the joint space, scavenges debris and free radicals, protects the chondral surface, creates flow barriers, and blocks capillary growth in the synovium [116–118]. While lower MW HA oligosaccharides promote the activation of iNOS and NO in bovine and human chondrocytes, HA with MW of 120 and 1260 kDa does not affect them [119]. Under oxidative injury, LMW Hyalgan exerts ameliorated damage and improved repair of mitochondrial DNA and preserves mitochondrial function and ATP levels to reduce mitochondrial-driven apoptosis, ultimately promoting chondrocyte survival in normal, injured, and osteoarthritic human knees [120,121]. HA with an LMW of 800 kDa mitigates NO production in the meniscus and synovium to protect against cell damage in rabbit OA models [116]. Similarly, HA with an LMW of 500–730 kDa blocks NO-induced apoptosis and dedifferentiation of rat chondrocytes [122]. Furthermore, HA with an HMW of 4000 kDa represses the increase in iNOS in mice with arthritis [123].

In bovine chondrocytes, Artz reduces ROS by regulating nuclear factor-erythroid-2-related factor 2 (Nrf2) by activating Akt, and 90 kDa MW HA improves proteoglycan synthesis and prevents IL-1-mediated cartilage degradation by inhibiting ROS/RNS, acting as an antioxidant to scavenge free radicals [124,125]. In calf chondrocytes, HA with a medium molecular weight of 2700 kDa reverses stress-inhibited proteoglycan synthesis to exhibit anabolic effects [126]. In addition to the restoration of IL-1β-induced decrease in proteoglycans, LMW Hyalgan reverses IL-1-induced increase in NO and PGE2, as well as the apoptosis triggered by the NO donor sodium nitroprusside (SNP) in human osteoarthritic chondrocytes of OA [117,127]. However, HMW Synvisc has no effect on basal production and IL-1-induced NO and PGE2, but reduces SNP-mediated apoptosis of human osteoarthritic chondrocytes treated with IL-1, indicating differences in biological activity depending on MW [117].

Fewer pain-eliciting nerve responses

The elastoviscous properties of HA exhibit analgesic and pharmacologic effects through interaction with HA receptors, joint nociceptors, and free nerve endings, as well as repression of the activity of stretch-activated mechanosensitive ion channels within cartilage tissue to mitigate the pain response in rat joints [128,129]. HA with higher MW of 860 and 2300 kDa exerts higher and longer-lasting analgesia than lower MW (> 40 kDa) HA by modulating HA receptors and masking free nerve endings of joint pain [128]. Different MW HA determines inhibitory effects on the frequency of evoked discharges in normal and inflamed joints: no reduction in both Hyalgan-treated joints, no effect in Orthovisc-treated normal joints, but a reduction in transient frequency reduction in inflamed joints; and a significant reduction (about 50%) in both Synvisc-treated joints [129]. To manage early osteoarthritic knees, Synvisc exhibits better pain relief and cost-effectiveness than Artz [130].

In a murine model, the crosslinked non-animal stabilized HA (NASHA) group with a vHMW of 100,000 kDa shows the most potent antinociceptive efficacy to reduce pain-associated behavior, followed by the crosslinked Synvisc One group, while the linear sodium HA group expresses the least effect [131]. Furthermore, HA suppresses human synovial cell proliferation to impede the expression of nociceptive pain mediators such as ARA, bradykinins, PGE2, and substance P, and these inhibitory effects are influenced by the MW of HA [132,133].

Superior cytoprotective effects

Chondroprotective effects are defined as functions that combine inhibition of cartilage degradation with the enhancement of HA, proteoglycan, and collagen synthesis, as well as the reduction of chondrocyte apoptosis. Exogenous HA impedes ROS/RNS production by decreasing NO and increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD) to reduce cartilage degeneration and chondrocyte apoptosis [134]. HA represses synovial cell degeneration and enhances Hsp72 expression by activating heat shock factor (HSF)1 and further inhibits cell death in stress cells to provide therapeutic benefit through chondral and subchondral protection [135]. By binding to CD44 through the reduction of MMP and ADAMTS expression, IA HA suppresses chondrocyte apoptosis to preserve vitality and maintains chondral and synovial integrity; however, it increases chondrocyte proliferation [108,109,121]. HA also binds to RHAMM to aid in chondroprotection [35,109].

The HMW (6000 kDa) HA, an indispensable component to maintaining articular joint homeostasis, exhibits higher chondroprotective effects and higher friction coefficients than the LMW (800 kDa) HA [136]. LMW Hyalgan, which does not affect spontaneous chondroptosis, binds to CD44 and ICAM-1 to inhibit anti-Fas induced chondrocyte apoptosis, but purified HA with MW 40–65 kDa does not, or HA at 1000 kDa causes a slightly smaller decrease, suggesting the importance of MW [137]. However, 0.5 mg/mL of Hyalgan shows significant increases in human normal chondrocyte survival, whereas

doses of 0.25 and 0.75 mg/mL only slightly increase cell viability [121]. In contrast, an inhibitory effect on chondrocyte viability is demonstrated at 1 mg/mL, indicating that dosage influences chondrocyte proliferation and survival in addition to MW [133]. Together, the degrees of anti-inflammatory, immunomodulatory, analgesic, and anti-OA effects of HA are determined by the dose and MW of HA.

Recommendations, systemic review, and meta-analysis of intraarticular hyaluronic acid

Since trials with 'negative' results tend to be unpublished and AEs can be minimally reported in industry-funded trials [138], it is imperative to reduce publication bias and skewed comparisons between

Table 2. Guidelines and recommendations for the treatment of KOA with IA HA in the century.

Reference	Organization/ committee	Guideline and recommendation
Jordan et al. [77]	EULAR	The higher MW product was significantly better at alleviating pain in a 12-week RCT 18 of 20 trials that assessed HA versus placebo were positive RCTs recorded significant reductions in pain compared to placebo for periods of 60 days to one year A study recorded functional improvements in the Lequesne index over one year
Dagenais [160]	CADTH	HA appears to be modest short-term pain reductions and functional improvements with rare, benign, temporary, and likely associated with IA AEs, and there is no superiority among HA products IA HA may be cost-effective, and most suitable for mild to moderate OA, and others are contraindicated or have failed
Nelson et al. [161]	COAMI	IA HA is controversial, receiving recommendations (but of low strength) or is recommended not to be used
Henrotin [158]	8 European experts	IA HA is effective and well-tolerated for mild to moderate KOA, but should not be used only in patients who have not responded adequately to analgesics and NSAIDs The dosing regimen must be supported by evidence-based medicine, and crosslinking HA is a proven means of prolonging IA residence time
Trojian [159]	AMSSM	Recommend IA HA for KL grade II-III KOA in those patients older than 60 years of age Suggest IA HA for KOA for those under the age of 60 years of age
Monticone [157]	SIMFER	Both IA HA and physical and rehabilitative interventions appear to be equally effective in improving disability, pain, and QOL in KOA
RACGP [84]	RACGP (2nd edition)	Not recommended to offer IA HA for KOA (a conditional recommendation against the procedure)
Kolasinski et al. [85]	ACR/Arthritis Foundation	IA HA is conditionally recommended against KOA because of no apparent benefits
Bannuru et al. [86]	OARSI	IA HA is conditionally recommended in individuals with KOA IA HA has beneficial effects on pain at and after 12 weeks and a more favorable long-term safety profile than repeated IA corticosteroids
Bruyère et al. [156]	ESCEO	A weak recommendation (56%) (<75% of votes were cast in favor of the 'strong do') of IA HA in patients who are contraindicated to NSAIDs, still symptomatic despite the use of NSAIDs, or at increased risk for NSAID-induced AEs IA HA is an effective treatment for KOA with beneficial effects on pain, function, and global assessment After 8 weeks after IA injection, HA shows superior and longer-lasting efficacy compared to corticosteroids A single injection of IA HA does not offer any benefit over the placebo, multiple injection courses are superior to a single injection (2−4 injections gave the largest effect size on pain at 3 and 6 months), and there appears to be no additional benefit given by a 5-injection course over a 3-injection course
NICE [162]	NICE	Do not offer IA HA for the management of OA [2014]
Phillips et al. [61]	CPGs	HA for KOA: 37.0% strong recommendations, 37.0% conditional recommendations, 7.4% uncertain recommendations, 7.4% weak recommendations against, and 11.1% strong recommendations against A general trend toward positive recommendations for HA between 2003 and 2020
Brophy et al. [155]	AAOS (3rd edition)	The calculated NNT was 17 patients Not recommended for routine use in the treatment of symptomatic KOA The strength of recommendation is moderate but alert to new information and sensitive to patient preferences

AAOS: American Academy of Orthopaedic Surgeons; ACR: American College of Rheumatology; AE: adverse event; AMSSM: American Medical Society for Sport Medicine; CADTH: Canadian Agency for Drugs and Technologies in Health; COAMI: Chronic Osteoarthritis Management Initiative; CPGs: clinical practice guidelines; ESCEO: European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases; EULAR: European Alliance of Associations for Rheumatology; HA: hyaluronan; IA: intraarticular; KOA: knee osteoarthritis; MW: molecular weight; NICE: National Institute for Health and Care Excellence; NNT: number needed to treat; NSAIDs: nonsteroidal anti-inflammatory drugs; OA: osteoarthritis; OARSI: Osteoarthritis Research Society International; QOL: quality of life; RACGP: Royal Australian College of General Practitioners; RCT: randomized controlled trial.

Table 3. Systemic review and meta-analysis of IA HA in the treatment of KOA.

Reference	SR/MA	Study (participant)	Conclusion and summary	IF (quartile) In 2021	Positive result
Lo et al. [100]	SR-MA	22	HA (Hyalgan, Suplasyn, Artzal, Orthovisc, BioHA, and Synvisc) has a small effect on the treatment of KOA, and the effect may be equivalent to the effect of NSAIDs on that of acetaminophen Compared to lower MW (615−3000 kDa) HA, the highest MW (Synvisc) HA is more effective in treating KOA	157.335 (Q1)	Yes *
Wang et al. [138]	SR-MA	20	Crosslinked HA (Synvisc) and non-crosslinked HA (Hyalgan, Artz, Orthovisc, and BioHA) decrease symptoms of KOA and improve pain and functional outcomes with few AEs Patients > 65 years of age and those with the most advanced radiographic stage of OA (complete loss of joint space) are less likely to benefit from IA HA	6.558 (Q1)	Yes
Arrich et al. [141]	SR-MA	22	HA (Hyalgan, Suplasyn, Artz, Sodium HA, Orthovisc, BioHA, and Synvisc, 500 to ~7000 kDa, most ~1000 kDa) has not been clinically effective and may have a greater risk of AEs	16.882 (Q1)	No
Pagnano et al. [140]	SR	14	Repeat courses of HA are safe and effective in the treatment of KOA pain	7.507 (Q1)	Yes
Bellamy et al. [139]	Cochrane Database of SR	40	IA HA is effective in treating KOA with beneficial effects: in pain, function, and global assessment, especially 5−13 weeks after injection IA HA is superior to placebo and comparable in efficacy to systemic forms of active intervention, with more local reactions but fewer systemic AEs, and more prolonged effects than IA corticosteroids	12.008 (Q1)	Yes
Reichenbach et al. [142]	SR-MA	13 (2085)	The likely lack of superior effectiveness of crosslinked hylan over HA (700−3000 kDa) and the increased risk of local AEs, including effusions or flares, associated with hylan No conclusions can be drawn on the effectiveness of IA HA compared to sham interventions	15.483 (Q1)	No †
Bannuru et al. [144]	SR-MA	7 (606)	In the short term (up to 4 weeks), IA corticosteroids are more effective than IA HA, whereas IA HA is more effective in the long term (4−26 weeks)	15.483 (Q1)	Yes
Bannuru et al. [87]	SR-MA	54 (7545)	HA for KOA pain is more than 6 months after injection, effective at 4 weeks, its maximum effectiveness at 8 weeks, and a detectable residual at 24 weeks The maximum effect size of HA is greater than that of other OA analgesics (acetaminophen, NSAIDs, and COX2 inhibitors)	7.507 (Q1)	Yes
Colen et al. [145]	SR-MA	74	HA improves pain by approximately 40−50% compared to baseline levels Unable to conclude that one brand has better efficacy than another due to the heterogeneity of the studies	7.744 (Q1)	Yes †
Rutjes et al. [146]	SR-MA	89 (12,667)	IA HA has a small and clinically irrelevant benefit and an increased risk of serious AEs	51.598 (Q1)	No

(*continued*)

Table 3 (*Continued*)

Reference	SR/MA	Study (participant)	Conclusion and summary	IF (quartile) In 2021	Positive result
Gallagher et al. [147]	SR	3	HA shows variable efficacy and is effective in lowering the cartilage loss rate in only 1 of 3 studies identified versus placebo	7.010 (Q1)	Yes
Bannuru et al. [88]	SR-MA	75 (4806)	IA HA is superior to NSAIDs in efficacy, possibly because of the integrated IA placebo effect, a small but robust difference IA HA shows a significant improvement from baseline pain, along with safety profiles and relative costs	51.598 (Q1)	Yes
Campbell et al. [148]	SR	14 MA (20,049)	Compared to other modalities, IA HA for KOA has improvements in pain and function up to 26 weeks IA HA has a good profile and should be considered in early KOA	5.973 (Q1)	Yes
Jevsevar et al. [149]	SR-MA	19	The clinical importance of outcomes involving pain relief and functional improvement does not support the routine use of HA Subdividing HA preparations by MW does not change the results of the analyses.	6.558 (Q1)	No †
Bannuru et al. [163]	SR-NMA	74 (13032)	Given the very low incidence of AEs, HA is relatively well tolerated and have a similar safety profile compared with each other	7.507 (Q1)	Yes
Johansen et al. [150]	SR-MA	71 (11,216)	IA HA shows a better effect than IA saline on pain reduction in OA	6.558 (Q1)	Yes
Zhao et al. [98]	SR- MA	20 (3034 patients and 3153 knees)	Synvisc and Synvisc One have almost the same pain relief effect for KOA as lower MW HA, but a superior effect favoring Synvisc and Synvisc One from 2 to 3 months after injection No increased risk of treatment-related AEs for Synvisc and Synvisc One	7.744 (Q1)	Yes *
Altman et al. [91]	SR	68	HA with MW $\geq$ 3000 kDa and those derived from biological fermentation have better efficacy and safety—factors influencing the selection of an IA HA product for KOA	7.010 (Q1)	Yes *
He et al. [151]	SR-MA	12 (1794)	IA corticosteroids are more effective in pain relief than IA HA in the short term (up to 1 month), while IA HA is more effective in the long term (up to 6 months)	13.4(Q1)	Yes
Gregori et al. [152]	SR-MA	17 (1651)	IA HA has greater benefits at 3- and 6-month follow-up	157.335 (Q1)	Yes
Ran et al. [44]	SR-MA	5 (1004)	HA is an effective therapy for KOA IA methylprednisolone shows comparable efficacy in reducing pain and improving functional recovery to HA, without a difference in long-term of AEs	13.4 (Q1)	Yes
Beaudart et al. [143]	SR-NMA	39 (3049)	6 months of HA treatment improves pain and/or physical function in KOA	11.431 (Q1)	Yes
Han et al. [82]	SR-NMA	38 (5262)	For pain relief and AEs, steroids are the best treatment for KOA, followed by HA (better than single PRP, multiple PRP, adipose MSC, and placebo)	5.973 (Q1)	Yes
Pavone et al. [103]	SR	6 guidelines	Most positive results are limited to IA higher MW HA, with a course of 2−4 injections a year	5.988 (Q1)	Yes *

(*continued*)

Table 3 (*Continued*)

Reference	SR/MA	Study (participant)	Conclusion and summary	IF (quartile) In 2021	Positive result
Singh et al. [83]	SR-NMA	23	HA decreases pain and improves function in KOA when compared with the placebo	7.010 (Q1)	Yes
Mojica et al. [49]	SR	79 (8761)	HA has good efficacy and is suitable for KOA but lacks the longevity	5.973 (Q1)	Yes

AE: adverse event; COX: cyclooxygenase; HA: hyaluronan; IA: intraarticular; IF: impact factor in Journal Citation Reports (JCR) 2021; kDa: kilodalton; KOA: knee osteoarthritis; MA: meta-analysis; MSC: mesenchymal stromal/stem cells; MW: molecular weight;; NMA: network meta-analyses; NSAIDs: nonsteroidal anti-inflammatory drugs; OA: osteoarthritis; PRP: platelet-rich plasma; SR: systemic review

* positive response to higher MW HA

† : negative response to higher MW HA.

trials [100]. The amalgamation of inconsistently defined 'higher' and 'lower' MWs and unequal injection numbers and doses could have blurred the benefits of IA HA, leading to confusion [44,139−154]. The latest edition from different organizations/committees is included in Table 2 (the previous editions are in supplementary Table A). Based on efficacy, safety, and cost-effectiveness, several recommendations and guidelines for IA HA for KOA were suggested. After adjustment for the same injection numbers, most of them were positive with a trend towards higher MW in selective patients with KOA [61,77,86,155−162].

An inadequate combination of heterogeneous trials or an inappropriate inclusion of low-quality studies, such as an incorrect indication of IA HA for patients who need surgical interventions for irreparably torn menisci or large (osteo)chondral defects, can lead to misleading conclusions from meta-analyses and therefore careful interpretation is required [98,163]. PubMed was searched from the database inception date to July 1, 2022, to focus on the efficacy of HA in KOA compared to placebo, NSAIDs, or corticosteroids in systemic reviews and meta-analyses and then to review their conclusions and consensus statements. Papers published in Q1 and having an impact factor > 5 are included in Table 3 (the others are collected in supplementary Table B). Despite efficacy without consistent results in different IA HA therapeutics, the current trend is toward higher MW preparations for a better strategy and fewer injection numbers [91,98,100−103,164−172].

Manufacturing vHMW HA

Nucleic acids and proteins possessing immunogenic effects in bovine vitreous and human umbilical cord HA are more abundant than those in rooster comb and bacterial HA [173]. As shown in Table 1, commercial HA is traditionally extracted from animal tissues, typically rooster combs, which contain high concentrations (7.5 mg/g) of HA (1200 kDa), and is more recently produced with the biotechnology of microbial fermentation using bacterial strains, especially *Streptococci* that can evade the host's immune system [174,175]. Moreover, the purity of production from cockscomb principally affects HA price and extremely high purity is required for injectable application in OA [174,176]. Even with healthy animal tissues and extensive purification, concerns about the wide range of sizes (polydispersity) and potential biological contamination of viruses (avian), protein residues (bovine), and nucleic acids causing possible allergic reactions reinforce the biotechnology of HA production [173].

Optimal microbial culture conditions can yield HMW HA (3500−3900 kDa), but contamination risks from pathogenic endotoxins and proteins are still evident [174]. Although the HA from rooster combs contains more endotoxins (23.0 EU) than bacterial technology (approximately 0.02 EU), the level of immunogenic effect of low protein residuals (1.0−1.6 μg/mg) in the bacterial HA could be higher than that (1.0 μg/mg) in the animal HA [173]. Concerning safety, endotoxin-free organisms with genetically modified recombinant strains, such as *Bacilli, Escherichia coli*, and *Lactobacilli*, may be alternative producers of HA [177,178].

HA can be chemically modified by conjugation and crosslinking through its two functional sites: the hydroxyl and the carboxyl groups to improve HA properties [174,177]. Conjugation grafts a monofunctional molecule onto the HA chain to increase function. Crosslinking recruits polyfunctional molecules to create a polymer network for increasing the MW and stiffness of HA and lowering the susceptibility of HA to enzymatic depolymerization. Synvisc One (hylan G-F 20), the first-ever single injection HA product, is a chemically crosslinked HMW HA containing hylan A and hylan B polymers made from chicken combs, and NASHA abolishes the utilization of animal parts during extraction of HA [176]. Through safe, nonpathogenic recombinant systems and chemoenzymatic technology to customize HA with defined vHMW and narrow polydispersity, the production of HA may be developed to avoid potential toxins [174,177].

A native form of vHMW (> 6000 kDa) HA, which is isolated from a naked mole rat of rodent species, exhibits superior cytoprotective properties than HA with regular higher MW in mouse, human, and other animal cells [112]. While vHMW HA decreases cell cycle arrest and death due to oxidative stress through CD44 in a polymer length-dependent manner by inhibiting the CD44 protein-protein interaction and at least partial modulation of p53 signaling, regular higher MW HA promotes them [42]. Therefore, vHMW HA at physical levels provides better protection of human cells from stress than the shorter HMW HA. Although it is still not clear whether vHMW HA is functionally different from conventional HMW HA, the implication of the anti-aging mechanisms of vHMW HA suggests potential applications for KOA.

Conclusion

To improve and customize its properties and applications, HA has been subjected to chemical modifications such as conjugation, crosslinking, and recombination. A variety of mechanisms involving receptors, enzymes, and various pathways, depending on MW, are involved in OA pharmacological and therapeutic effects, regardless of whether they are avian-derived or biofermentation-derived. After the high-quality bioprocessing of vHMW HA production, a significant reduction in injections and improved joint function will contribute to improved economic cost-utility and the potential for disease-modifying interventions. The selection of HA and the indication of use are of the utmost importance. Tremendous mechanical stress due to a progressive meniscal tear or (osteo)chondral ulcer triggers the vicious cycle, which must be operated with excellent techniques to reverse as soon as possible. To decrease side effects and increase efficacy and duration of action, clinicians should also be aware of the derived differences and MW in the manufacture of the various available HA products.

Funding

This article received no funding.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Code availability

Not applicable.

Author contributions

All authors work on content and writing collaboratively.

Data availability

No data was used for the research described in the article.

Conflicts of Interest/Competing Interests

None

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.matbio.2023.02.006.

Received 3 January 2023;
Received in revised form 8 February 2023;
Accepted 21 February 2023
Available online 26 February 2023

Keywords:
Hyaluronan;
Inflammation;
Molecular weight;
Osteoarthritis

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