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Low Molecular Weight Hyaluronic acid Mechanism of action

A collection of evidence on how Low Molecular Weight Hyaluronic Acid is able to enhance endogenous Hyaluronic Acid in the case of Osteoarthritis

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The concept of Low and High Molecular Weight could be very different depending on the prospective views:

	Low Molecular Weight	High Molecular Weight
Scientific/laboratory prospective	10 K to 1MDa	20K to 2MDa
Clinical publication view	??? ~ Not always specified. ~ Standard point of reference is the number of injections which is also often missed out. ~ Important to check who is the sponsor.	
Manufacturing	<1.2 MDa	>2MDa

This is important especially when you are comparing scientific journals to the ones on clinical evidence and then comparing it to products available on the market.

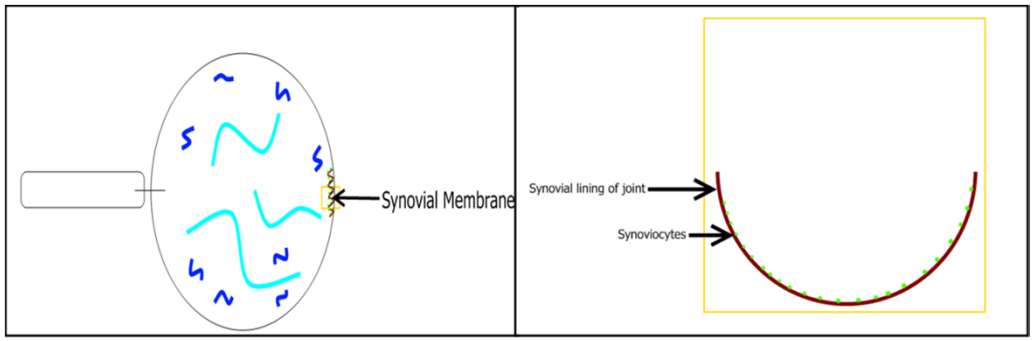
LMW HA impact on Synoviocytes:

Stimulate production on endogenous HA: Mechano-Sensitive Effect (MSS)

Components involved

- Synovial Membrane
- β-synoviocytes (cells found on the synovial membrane which produce endogenous HA)

Each synovial joint is surrounded by a highly vascular capsule called synovium, whose internal surface layer is lined with a synovial membrane. Inside this membrane, type β-synoviocytes are localized.



LMW implication in Mechano-Sensitive Stimulation

- Increases volume of HA in synovial joint
- Increases hydrodynamic volume in synovial joint
- β-synoviocytes of the synovial membrane respond to mechanical deformation (i.e. stretching of the synovial membrane)

LMW action: Increase (endogenous) HA production providing a long-lasting effect

Activation of β-synoviocytes: Importance of HA

Components involved in a compressed Synovial membrane

- LMW HA
- β-synoviocytes

LMW HA diffuses (rapidly due to its size) and attaches to the β-synoviocytes receptors, promoting endogenous production of HA.

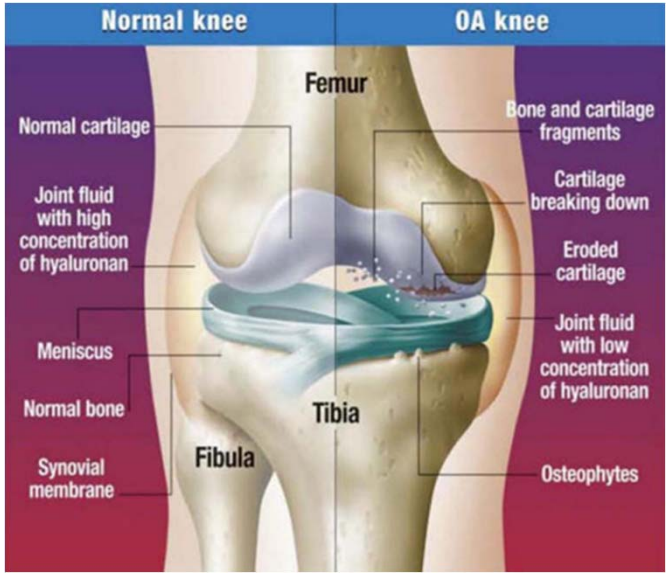
Healthy Knee Joint Vs. OA Joint

- In a healthy knee joint **β-synoviocytes are easily accessible** → **Continuous production of HA.**

- In OA joint: the synovial membrane is "folded". As the β-synoviocytes cells rest on the synovial membrane, due to this folding:

  – Large HA are **limited in gaining access**, thereby limited in activating the β-synoviocytes.

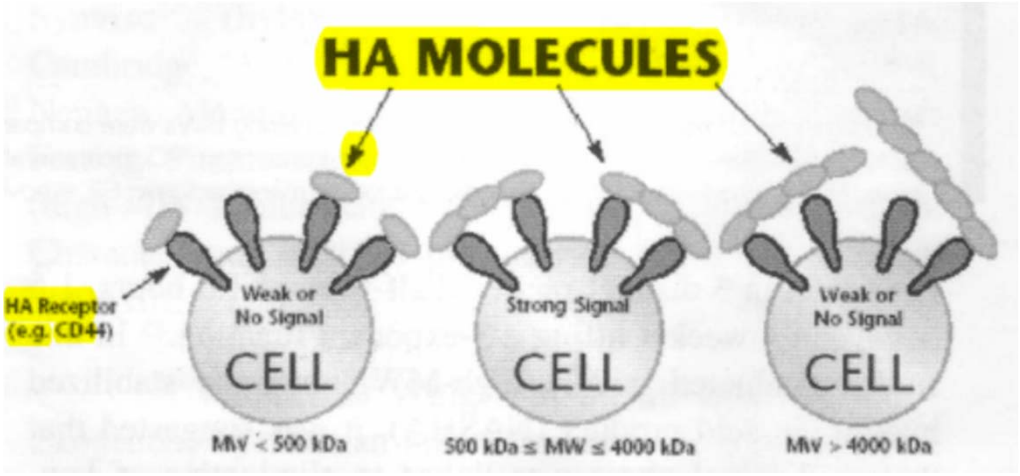
  – **Smaller HA molecule** (i.e. LMW HA) purely due to its size, will be able to **gain easier access** to the β-synoviocytes and re-stimulate the production of endogenous HA.



Literature extracts

Quotations illustrating that LMW HA can penetrate easier (than HMW) to the β-synoviocytes to stimulate production of endogenous HA:

- "Low to mid MW hyaluronans (ie. 840kDa) penetrate diseased tissues more effectively that high MW hyaluronans (ie. 2300kDa)" [1]
- "Synovial fibroblasts do not increase synthesis of endogenous hyaluronans in the presence of functionally acceptable (ie very HMW or high concentration) hyaluronans"[1]
- "Beyond a certain limit, very large hyaluronan molecules will be less efficient in engaging multiple receptors because of the steric hindrance – hence the bimodal nature of many biological activities. This suggests that the maximal response would be produced by hyaluronans within a specific size range (neither too big nor too small)" [1]



- "Synovial fibroblasts derived from an osteo-arthritic joint demonstrated the most marked response on exposure to exogenous HA, showing a stimulation of HA synthesis with preparations of weight-average molecular weight (MW)>$5x10^5$ in a concentration dependent manner" [2]

- "Stimulation of HA synthesis was produced by HAs with MWs >0.5 X 10^6 Da ·…· The cell response appeared to decrease when HAs of MW > $3x10^6$ – $4x10^6$ Da were used. The authors hypothesized that HA synthesis was mediated by interaction of this GAG with surface receptors on these fibroblasts that only promoted a signal for increased by binding to HA within a certain molecular size range" [5]

Quotations illustrating the long lasting effect of LMW HA:

- "Longer lasting clinical benefits can only be reconciled by acceptance that this agent (HA) is capable of pharmacologically modifying certain pathologic pathways in OA that give rise to symptoms. In this regard, the partial restoration of synovial cell metabolism and normalization of HA biosynthesis ("viscoinduction"), which has been shown in both animal models and human subjects using HAs within the MW range of $0.5x10^6$ – $1.0x10^6$ Da may be of considerable importance" [5]

- "An interesting effect of 500-730 kDa HA is the stimulation of endogenous HA synthesis by synoviocytes *in vitro*. It has been shown that synovial fibroblasts obtained from knee joints of patients with OA synthetized hyaluronic acid at a lower rate than cells derived from normal synovia. These fibroblasts respond to the presence of 500-730 kDa HA by increasing the biosynthesis of hyaluronic acid in a concentration dependent way. Once initiated such a process may be self-sustaining and may explain the prolonged effect of the substance" [7]

LMW HA role in Proteoglycan formation by protection of Chondrocytes

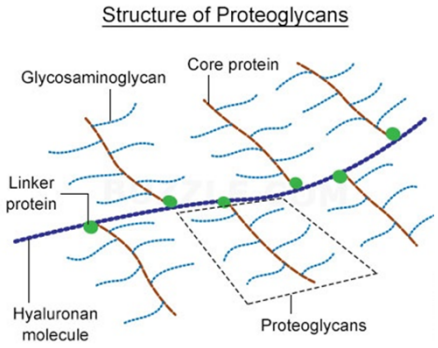
Components involved

- Cartilage (Chondrocytes)
- Proteoglycans

Proteoglycans are a major component of the extracellular matrix, the "filler" substance existing between cells in an organism.

Chondrocytes are the only cells found in healthy **cartilage (*ECM).** They produce and maintain the cartilaginous matrix, which consists mainly of collagen and proteoglycans.

*ECM: Extra Cellular Matrix



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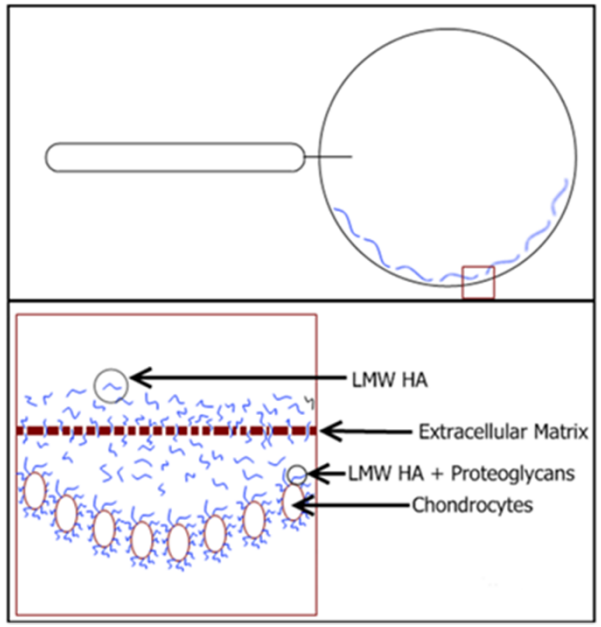
LMW HA defined action



LMW HA Actions

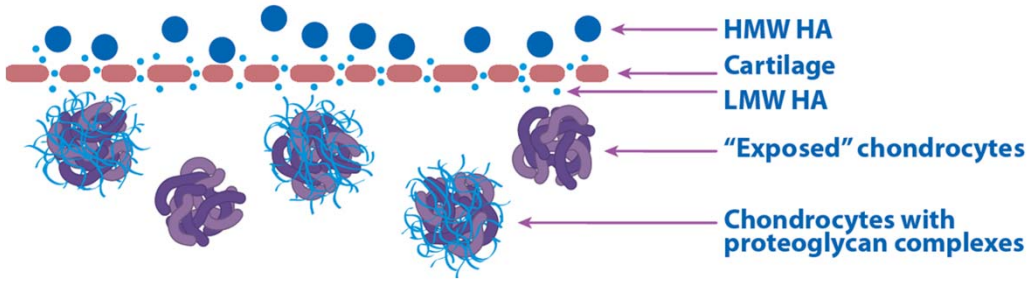
- Diffusion through cartilage and ECM of chondrocytes.
- Reaction other molecules (proteins) to form proteoglycans which then form a protective cage around chondrocytes
- Reduction of chondrocytes apoptosis
- Increase production of endogenous HA which is long lasting

*ECM: Extra Cellular Matrix

Proteoglycan formation by protection of Chondrocytes

- Diffusion of LMW HA and its absorption on the cartilage surface is facilitated as the permeability through the cartilage is low - (LMW HA, has a higher rate of penetration.)

- LMW HA (protective ability) - "caging effect": Chondrocytes found within the cartilage are protected by proteoglycans (which are molecules that require HA as a structural backbone component).



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Literature extracts

Quotations illustrating that LMW HA is able to reduce chondrocytes apoptosis:

- "Mid-MW hyaluronan (500-999 kDa) appear to inhibit apoptosis more, offer more protection against cartilage loss and suppress synovial cell proliferation more" [1]

- "Effect of HA preparations on chondrocytes apoptosis induced by nitric oxide: ·…· The 6,000 kDa HA decreased apoptosis by 36% and the 500-730 kDa decreased apoptosis by 40%"[4]

- "HA (500-730 kDa), which is the major component of the extracellular cartilage matrix, can protect chondrocytes against proteoglycan depletion and cytotoxicity induced by IL-1 and oxygen-derived free radicals. As shown by this study, HA can also exert an antiapoptotic effect on IL-1 stimulated chondrocytes" [6]

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Quotations illustrating that LMW HA will better (than HMW) foster cartilage integrity:

- "Hyaluronic acid has a significant stimulatory effect on the metabolic activity of chondrocytes that may provide an explanation for the longer term clinical benefits. Studies on osteoarthritic cartilage have shown that hyaluronic acid depletion in the extracellular matrix occurs before structural changes in proteoglycans are detectable"[3]

- "It has been demonstrated that 500 KDa hyaluronan is able to reduce such a ratio in chondrocytes cultured for 8 days in presence of IL1ß, suggesting a possible mechanism for the maintenance of cartilage integrity after treatment" [7]

- "In animal models of OA, HAs with MW $>2x10^6$ were reported to be less effective to cartilage and synovial cell metabolism than HAs of less than half this size"[5]

HA effect: Lubrication

During inflammation - OA:

- HA removes free radicals
- Aids in the preservation of SAPLs (radicals which attack Surface Active PhospoLipids)
- LMW HA, contains shorter chains which favour less degradation of O2 free radicals during inflammation process, hence less structural changes[8].

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Literature extracts

- "HA preparations with MWs within the range of 500-1000 kDa were generally more effective in reducing the indices of synovial inflammation and restoring the rheological properties of SF than HA preparations with MW> 2,300 kDa"[4]

- "In vitro study showed that HA of 0.7 x 106 Da MW inhibited leukocyte migration, chemotaxis and adhesion in a concentration dependent manner. Forrester and Wilkinson showed a reduction on binding of chemotactic factors to the cell surface in the presence of HA. […] It was proposed that the effects of HA on the activated neutrophils were via interation with their CD44 cell receptors" [5]

- "HA fractions with MW <1.0 x 10^6 Da are capable of modulating the response of articular chondrocytes to inflammatory mediators in vitro"[5]

- "HA (500-730 kDa), which is the major component of the extracellular cartilage matrix, can protect chondrocytes against proteoglycan depletion and cytotoxicity induced by IL-1 and oxygen-derived free radicals. As shown by this study, HA can also exert an antiapoptotic effect on IL-1 stimulated chondrocytes"[6]

- "One major action of HA after injection into the joints could be the capturing of inflammatory mediators within the synovial fluid, thereby leading to beneficial effects to the chondrocytes"[6]

- " The products of hyaluronidase degradation of HA, apart from chain length, have a chemical structure that is identical to that of the parent polymer. However fragments generated by chemical means differ greatly from the parent chain. The products of O_2 metabolism are incorporated into the chains and modify their structure. Such HA fragments contain aldehydes and hydroperoxides at their reducing termini. Both are extremely reactive" [8]

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