

# Hyaluronan viscosupplementation: state of the art and insight into the novel cooperative hybrid complexes based on high and low molecular weight HA of potential interest in osteoarthritis treatment

Chiara Schiraldi  
 Antonietta Stellavato  
 Francesca de Novellis  
 Annalisa La Gatta  
 Mario De Rosa

Department of Experimental Medicine Section of Biotechnology, Medical Histology and Molecular Biology, Second University of Naples, Bioteknet, Naples, Italy

Address for correspondence:

Mario De Rosa

Department of Experimental Medicine Section of Biotechnology, Medical Histology and Molecular Biology  
 Second University of Naples, Bioteknet  
 Via L. De Crecchio 7  
 80138 Naples, Italy  
 E-mail: mario.derosa@unina2.it

## Summary

**Osteoarthritis (OA) represents a group of chronic, painful, disabling conditions affecting synovial joints. It is characterized by degeneration of articular cartilage, alterations of peri-articular and subchondral bone, low-grade synovial inflammation (synovitis). Despite OA is commonly described as a non-inflammatory disease, it is known that its progression and the subsequent increment of symptoms correlate to the production of inflammatory factors that induce the secretion of enzymes responsible for cartilage degradation. In clinical practice, to alleviate pain and stiffness, not only during acute phases but also as maintenance therapy, intra-articular injections of corticosteroids or similar drugs are used, besides it is well diffused the viscosupplementation procedure based on hyaluronan gel. There are many different products containing high molecular weight linear HA or cross-linked derivatives, however the novelty in the field consist in the hybrid cooperative complexes derived from high and low molecular weight HA through a patented processing. This technique permit to double the amount of HA delivered to the injured site without increasing the injected volume, beside *in vitro* assay on human chondrocytes suggested hybrid complexes as effective in the modulation of several inflammatory cytokines in joints.**

**KEY WORDS:** osteoarthritis; glycosaminoglycans; hyaluronan; cytokines; viscosupplementation; cooperative hybrid complexes.

Hyaluronan (HA) and other glycosaminoglycans (GAGs), naturally present in the extracellular matrix, play an important role in the cartilage functions and joints. HA is a het-

eropolysaccharide consisting of tandem repeats of D-glucuronic acid and N-acetyl glucosamine and is abundantly present in the synovial fluid, where it can help to lubricate joints toward a smooth motion (1). Osteoarthritis (OA), also known as a degenerative joint disease, is characterized by a gradual loss of cartilage. However there are other important contributing factors, including obesity (which increases mechanical stress), history of joint trauma or repetitive joint use, genetics (innate and acquired), metabolic disorders, muscle weakness, underlying anatomical and orthopedic disorders (e.g. congenital hip dislocation), joint infection, crystal deposition, and a variety of bone turnover and blood clotting disorders (1, 2). Recently, scientific interest is beginning to focus on associations with sex hormones, obesity, and physical activity to determine whether factors including estrogen, weight management and protection during sport and exercise can be used as treatment for postmenopausal women with OA. Also the latter has an important inflammatory component due to several cytokines production in synovial fluid (3). These inflammatory factors drive the production and secretion of metalloproteinases, collagenases, and similar hydrolytic enzymes that mediate destruction of aging cartilage. In current years, the only clinical therapy applied is represented by non-steroidal anti-inflammatory drugs (NSAIDs) (4). Intra-articular injection of corticosteroids-like molecules may alleviate both pain and stiffness, not only during acute phases (episodic pain peaks) but also as maintenance therapy (5). Considering the increment of patients around the world, effective and reliable solutions are needed as alternative to traditional treatments. Since, it is reported that people with OA have lower levels of hyaluronic acid in their synovial fluid, currently, viscosupplementation (4) is an established medical concept to restore the rheological features and homeostasis into synovial fluid of osteoarthritic joints through the injection of hyaluronic acid at different doses (6). Generally the major drawbacks of linear HA exploitation is its short half-life *in vivo* rapid degradation due to enzymatic attack, but also to free radicals and other mechanisms (5). For this reason, scientific community aimed to design chemically cross-linked HA (7) for improving its *in situ* permanence, providing long-term stability. Composite of CS-alginate-hyaluronan have been evaluate as scaffold for the development of cartilage regeneration. *In vitro* experiments showed neo cartilage formation (8) while implanted scaffold led to partial repair of cartilage defect *in vivo* (9). *In vitro* studies proved that cross-linked HA is more stable to BTH (bovine testicular hyaluronidases). In fact, the Authors reported that even HA formulations with a low cross-linking degree needed 10-fold of BTH amounts to complete degradation (6). It is widely reported that biochemical alterations caused by OA involve in the mechanical alterations of the synovial fluid specifically rheological and viscoelastic properties. In fact, both HA molecular weight and concentration decrease, hamper cartilage functionality consequently resulting into pain and even disability (10, 11). The viscous and elastic behavior of hyaluro-

nan solutions have been studied to better address the pathological treatments. The first studies of Balazs et al. (12) introduced rheological parameters to compare different preparations/formulations, towards better indications of *in vivo* applications. In particular the visco-elastic properties of synovial fluid are described by the elastic modulus ( $G'$ ) and viscous modulus ( $G''$ ) as a function of frequency (13). Synovial fluid should provide the required viscoelastic response to compression and tangential forces arising in everyday life, allowing cartilage–cartilage contact and increasing wear of the joint surface. Several factors affect the viscosity and thus lubricant function of this anionic molecules in their hydrated state, such as pH, chain length, molecular weight, cross-linking degree, and chemical modifications (11). Intra-articular treatment with un-cross-linked HA and cross-linked HA respectively, are widely diffused nowadays (14). However, despite generally encouraging outcomes, mechanical stimulation is one of the major regulators known to maintain *in situ* cartilage integrity and function. In particular, specialized bioreactors are capable of reproducing a joint-like surrounding thank to compression, shear and fluid flow. According to the Authors (10), the mechano-transduction plays a pivotal role in synovial joints, thus the more the system may reproduce joint kinematics, the more robust will be the predictive outcome *in vivo*. In the last years, literature reports different clinical studies focused on OA and HA treatment. In fact clinical trials have demonstrated the efficacy and tolerability of intra-articular HA for the treatment of pain. For example, Migliore et al. reported that three injections of commercial cross-linked HA can reduce knee pain up to 6 months (6). Taken together these considerations, we could highlight that numerous papers referred to cross-linked hyaluronan: water-soluble carbodiimide cross-linking, polyvalent hyadrazide cross-linking, divinyl sulfone (DVS) cross-linking, disulfide cross-linking and photo-crosslinking of hydrogels through glycidyl methacrylate-HA conjugation have been introduced for tissue engineering applications of HA and are available as viscosupplements for intra-synovial injection (15, 16). Nevertheless, a concern is rising on the use of chemically modified hyaluronan (13), beside there is growing scientific emphasis for modulation of HA activity based on its molecular weight (15). In this respect, hybrid cooperative HA complexes, produced through a patented technology, represent a new and valuable alternative, permitting to deliver the double of the HA amount in the same volume with a contained and even reduced dynamic viscosity. In particular, D'Agostino et al. (2015) (17) reported the efficiency of hybrid complexes (H-HA; MW  $1200 \pm 200$  kDa and L-HA: Mw =  $100 \pm 5$  kDa) molecular weight hyaluronic acid on a scratch *in vitro* model. Interestingly, H-HA/L-HA hybrid complexes improved the reparation process compared to control and even HHA alone. In our preliminary *in vitro* studies, innovative complexes reduced inflammation in primary chondrocytes proving the reduction of specific biomarkers such as TNF- $\alpha$  and IL-6 analyzed at transcriptional (quantitative Real Time PCR) and protein level. These innovative hybrid cooperative HA com-

plexes due to their outstanding biochemical and biophysical features and to the remarkable biological action could represent a valuable alternative to cross-linked HA for different biomedical device applications.

## References

1. Dicker KT, Gurski LA, Pradhan-Bhatt S, Witt RL, Farach-Carson MC, Jia X. Hyaluronan: a simple polysaccharide with diverse biological functions. *Acta Biomater.* 2014 Apr;10(4):1558-70.
2. Moreland LW. Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: mechanisms of action. *Arthritis Res Ther.* 2003.
3. Scanzello CR, Umoh E, Pessler F, Diaz-Torne C, Miles T, Dicarlo E, Potter HG, Mandl L, Marx R, Rodeo S, Goldring SR, Crow MK. Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease. *Osteoarthritis Cartilage.* 2009 Aug;17(8):1040-8.
4. Balazs EA, Denlinger JL. Viscosupplementation: a new concept in the treatment of osteoarthritis. *J Rheumatol Suppl.* 1993;39:3.
5. Watterson JR, Esdaile JM. Viscosupplementation: therapeutic mechanisms and clinical potential in osteoarthritis of the knee. *J Am Acad Orthop Surg.* 2000;8:277.
6. Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. *Acta Biomater.* 2013.
7. La Gatta A, Schiraldi C, Papa A, D'Agostino A, Cammarota M, De Rosa A, De Rosa M. Hyaluronan scaffolds via diglycidyl ether crosslinking: toward improvements in composition and performance. *Carbohydr Polym.* 2013.
8. Lahiji A, Sohrabi A, Hungerford DS, Frondoza CG. Chitosan supports the expression of extracellular matrix proteins in human osteoblasts and chondrocytes. *J Biomed Mater Res.* 2000 Sep 15;51(4):586-95.
9. Burdick JA, Chung C, Jia X, Randolph MA, Langer R. Controlled degradation and mechanical behavior of photopolymerized hyaluronic acid networks. *Biomacromolecules.* 2005 Jan-Feb;6(1):386-91.
10. Wimmer MA, Grad S, Kaup T, Hänni M, Schneider E, Gogolewski S, Alini M. Tribology approach to the engineering and study of articular cartilage. *Tissue Eng.* 2004 Sep-Oct;10(9-10):1436-45.
11. Kurisawa M, Chung JE, Yang YY, Gao SJ, Uyama H. Injectable biodegradable hydrogels composed of hyaluronic acid-tyramine conjugates for drug delivery and tissue engineering *Chem Commun (Camb).* 2005.
12. Balazs EA, Laurent TC, Jeanloz RW. Nomenclature of hyaluronic acid. *Biochem J.* 1986.
13. La Gatta A, Papa A, Schiraldi C, De Rosa M. Hyaluronan dermal fillers via crosslinking with 1,4-butandiol diglycidyl ether: Exploitation of heterogeneous reaction conditions. *J Biomed Mater Res B Appl Biomater.* 2016.
14. Conrad BP. The effects of glucosamine and chondroitin on the viscosity of synovial fluid in patients with osteoarthritis. *Citeseer;* 2001.
15. Kim ZH, Lee Y, Kim SM, Kim H, Yun CK, Choi YS. A composite dermal filler comprising cross-linked hyaluronic acid and human collagen for tissue reconstruction. *J Microbiol Biotechnol.* 2015.
16. Allison DD, Grande-Allen KJ. Review. Hyaluronan: a powerful tissue engineering tool. *Tissue Eng.* 2006.
17. D'Agostino A, Stellavato A, Busico T, Papa A, Tirino V, Papaccio G, La Gatta A, De Rosa M, Schiraldi C. In vitro analysis of the effects on wound healing of high- and low-molecular weight chains of hyaluronan and their hybrid H-HA/L-HA complexes. *BMC Cell Biol.* 2015.