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Engineering of Viscosupplement Biomaterials for Treatment of Osteoarthritis: A Comprehensive Review

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Osteoarthritis (OA) is a progressive degenerative disease that causes severe pain and functional limitation, especially during locomotion. It is the most common arthritis type that damages the surface of articular cartilage until the underlying bone. In the past decade, the scientific community has made a considerable effort to improve or discover therapeutical products used as a form of conservative treatment capable of restoring the damaged articular tissue, avoiding, as far as possible, the use of surgical practices. The most common and direct nonoperative application available for OA treatment is the viscosupplementation (VS) procedure that demonstrates a safe, effective method and is less painful for the patients. The most recent works dealing with the design, development, and validation of viscosupplement products in preclinical and clinical trials for OA treatment are overviewed herein. In general, despite the development of new products, hyaluronic acid continues to be among the most reported intra-articular viscosupplement products used in clinical trials, typically used as an isolated product or conjugated with other biologicals or drugs, such as platelet-rich plasma and corticosteroids (CS). However, this issue is still demanding innovation. Approaches comprising new biomaterials as VS products, with intrinsic bioactivity, economical, and environmental friendliness, are required.

1. Introduction

The function of the musculoskeletal system of the human body is mainly related to movement. Bone is the key load-bearing element of this system, which also comprises skeletal muscles and connective systems, providing protection and mechanical support/stability to the body.[1] Bones function as rigid devices articulating with each other throughout joints, using the ligaments. In contrast, the skeletal muscles work as contractile devices connected to the bone by the tendons.[2]

Bone is hierarchically organized to achieve its purposes: protect vital structures, mechanical support, metabolic and endocrine, and hematopoietic (generation of blood cells) support.[3] Bones ensure high tensile and compressive strength but also give elasticity.[4] Another bone function is related to storage as they offer a storehouse of calcium, phosphorus, and other minerals.[4] It is frequently defined as a composite material, with organic and inorganic constituents, with calcium phosphate apatite nanocrystals (hydroxyapatite) inserted in the collagen matrix.[5] It is a very dynamic and metabolically active living entity, with its specific vascular supply and innervation,[3] being also exceptionally lightweight and a robust tissue.[4] The process of bone growth, or bone remodeling/metabolism, occurs through a lifetime and with different rates, comprising removing and replacing old with new bone tissue, respectively.[5b] Thus, there is controlled synchronization between bone-building cells and bone-resorbing cells, namely, the removal of mineralized bone by osteoclasts, accompanied by bone matrix's growth by osteoblasts, which later become mineralized.[6]

The cartilage is an assembly of tissues with low vascularity and is composed of cells enclosed by a specialized extracellular matrix containing essentially collagen (type II) and proteoglycan. Cartilage is one of the two central tissues of the skeleton (the other is bone), and it can be found in several human body regions (e.g., joints, ear, nose, and throat), accomplishing several proposals: framework, covering surfaces, and absorbing impacts.[7] Thus, its primary functions are mechanical impact/shock absorption, joint motion assistance, structural support, and connection between soft and hard tissues.[8] However, besides these essentially mechanical functions, cartilage also has a unique and notable role: it is responsible for forming a model for the later development of the human skeleton. Accordingly, in the embryo, the chondrocytes are replaced by osteocytes or other bone cells.

Moreover, while growing, a thin cartilage plate (epiphyseal plate) remains at the bones' edges. This allows the production of new bone while osteocytes substitute the chondrocytes. This bone-growing process finishes once this plate disappears.[9]

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Cartilage can be clustered into three major groups: 1) elastic cartilage, 2) fibrous cartilage (or fibrocartilage), and 3) hyaline cartilage (**Figure 1**).

The collagens and proteoglycans' amount, distribution, and types are distinct for each cartilage type.[10] The elastic cartilage is located in nonweight-bearing body parts (such as epiglottis, external ears auricle, auditory tube, and nose).[11] It usually does not endure high mechanical loads for a long time. It can tolerate distortion and return to its original shape. This structural cartilaginous tissue, in contrast with the others, usually does not have significant medical problems. Fibrocartilage type of cartilage is the most inflexible and very rigid tissue. It can be subdivided into 1) intra-articular (IA), 2) connecting, 3) stratiform, and 4) circumferential fibrocartilage, depending on their distinct functions. Fibrous cartilage can be found mainly in the intervertebral disks and at the insertions of ligaments and tendons into the bone.[12] The most frequent type of cartilage is hyaline cartilage, mainly present on the synovial joints to reduce friction and prevent bone injury during the movement. It can also be found in the trachea and nasal septum or bones' interior and embryo skeleton development.[7] The elasticity is an essential parameter for the function of each cartilage type. For instance, the cartilage for the ears and nose has more elasticity (elastic cartilage). Thus, the cartilage should have a particular degree of elasticity to allow higher flexibility or strength to accomplish its specific roles. For example, for medial and lateral menisci of the knee joint, the cartilage must have tensile strength to support the high mechanical stress of movement and weight, having lowest elasticity.[13]

The articular cartilage is precisely adjusted to withstand the movement's dynamic compression load and shear force inside every synovial joint. The cartilage's inherent repair capacity is shallow.[14] Thus, injuries and age-related articular cartilage degeneration often cause significant pain and disability. This problem is more prominent and growing, considering a globally aging population. Cartilage damage is a notable characteristic of degenerative joint[15] disorders, and extracellular matrix (ECM) damage is a common occurrence of most diseases affecting cartilage.

The challenges associated with the treatment of cartilage diseases are related to the lack of knowledge on their pathogenesis and etiology and the symptoms that typically occur after substantial ECM structural destruction as cartilage is aneural. Moreover, the known lack of vascularity and the high ECM density create difficulties in drug transport, leading to challenges in treating cartilage diseases.[10] The dense packing of ECM components also hinders the transport of drug molecules in the tissue, which, along with the lack of vascularity, poses an additional challenge to treating cartilage diseases.

OA is one of the most prevalent musculoskeletal disorders worldwide. It is strongly associated with age (more recurrent after 40s), a leading cause of chronic debility for older people. OA incidence is similar for males and females and represents a significant cause of morbidity, limitation of activity, and overall healthcare utilization. The number of people affected globally increased by 48% from 1990 to 2019,[10] affecting almost 10% of the worldwide population and 6% of the European population, resulting in substantial clinical, humanistic, and economic burden. In addition, OA directly affects managing other chronic conditions, such as diabetes, heart disease, and hypertension, increasing the OA burden. It is a leading cause of incapacity and illness problems in high-income countries.[16]

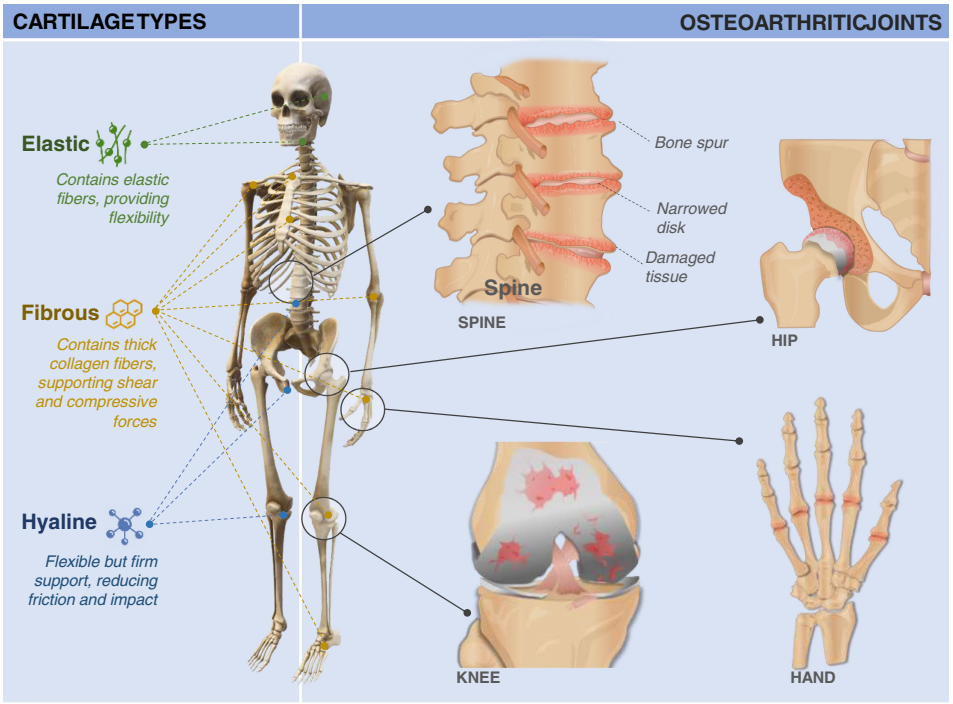

Figure 1. Cartilage types and location in the human body (left side) and the most frequently affected joints by osteoarthritis (OA) disease (right side).

Moreover, the primary trigger of disability among the elderly population is kneeOA. The joints most affected are the knee, hip, and shoulder, being also generally detected in the feet and hands. Although symptomatic OA can comprise single and multiple peripheral joints, the most common joint affected is the knee.[17] The global prevalence of knee OA is 22.9% in people older than 40 years.[18] The osteoarthritic hip and knee are associated with moderate-to-severe disability, even in young adults. The hip and knee OA estimated prevalence in European countries is 35.0%, among people aged 50–59 years, and 55.0% over 70 years of age.[19] The lifespan risk of developing symptomatic knee OA is estimated to be about 45.0%, increasing to 60.5% for particular cases (e.g., obesity).[20]

The economic impact of OA is high, with the average total annual cost per patient in Europe from 1330€ to 10 452€.[21] Globally, the weighted average annual costs per patient (until 2014) with knee and hip OA were 11.1, 9.5, and 4.4 k€ for total, direct, and indirect costs, respectively.[22] Besides being very substantial, such values will continue to rise, being undoubtedly assumed that the indirect costs in working patients are much higher.

Pain is the main symptom of OA, being the main reason why affected individuals seek medical care. Following pain, the most noticeable symptoms include morning stiffness, usually short duration, accentuated by movements and reduced by rest.[23] Hidden behind these symptoms, as the disease progresses, the joints experience severe degeneration of cartilage, narrowing of the space, subchondral bone thickening, formation of osteophytes or bone spurs, and inflammation in the joint.[24] The treatment options comprise weight management, physical activity, medications, joint replacement surgery, and various other techniques, mainly to alleviate the symptoms, being very challenging to restore normal cartilage function.[25] New tissue engineering (TE) approaches have shown to be promising to regenerate damaged articular cartilage and help regain normal body functions. Specialists have traditionally used IA administration of hyaluronic acid (HA) (viscosupplementation [VS]) for patients suffering from degenerative joint diseases.[26]

This review presents an overview of cutting-edge knowledge regarding the advances in the development of novel viscosupplements, along with the therapeutic considerations of those. It is also reviewed the recent preclinical works and current clinical trials.

2. Biomaterials and Viscosupplement-Based Therapies

The treatment and management of knee OA usually follow a pyramidal sequence.[27] It essentially follows reasoning based on symptoms (e.g., pain and knee function), the stage of the disease, and other patient-linked aspects (e.g., age, physical activity, and comorbidities).[28] Briefly, the primary treatments are conservative methods, such as pain control, muscle strength, and conditioning focusing on education, adequate exercises, and weight control. Then, to a small number of patients, pharmacological treatments are applied. After this, more invasive solutions are usually used[29] (**Figure 2**). Accordingly, the Osteoarthritis Research Society International (OARSI) regulations for managing the knee, hip, and polyarticular OA initially indicate the proper utilization of conservative/nonsurgical treatments (physiotherapy, weight reduction, VS, pain relief, anti-inflammatory drugs). When the symptoms persist, surgical procedures (such as unicompartmental or total knee arthroplasty [TKA], osteotomy, cartilage restoration, and arthroscopic debridement) are considered.[30]

Unicompartmental/partial knee arthroplasty (UKA) or replacement had a favorable evolution of clinical results in the past years with outstanding success rates of modern implants, mainly due to the minimally invasive surgery needed and the growth and refinement of surgical procedures and implant design. The surgical method of UKA relatively to TKA has some benefits associated with faster healing, less pain following surgery, and blood loss, as well as the patients reporting it as a gentler process as the healthy parts of the knee is kept.[31] In contrast, TKA provides trustworthy effects for end-stage, tricompartmental, and degenerative OA. It is one of the most profitable and regularly successful orthopedics' surgeries. This procedure allows the patient to have pain relief and knee function restored, leading to an enhanced quality of life.[32] The knee osteotomy consists of a surgery that reshapes a part of the tibia and femur bones for axis correction to relieve pressure on the joint. These are pioneering and efficient therapeutic procedures for early-stage and unicompartmental knee OA handling.[33] This procedure works by transferring the load off the joint-injured side, relieving pain and significantly improving function.

The surgical approaches to repairing articular cartilage are bone marrow stimulation (BMS) and osteochondral autografts. BMS treats focal articular cartilage defects by a microfracture procedure, usually performed arthroscopically, which creates a fibrocartilaginous fill of pluripotent marrow cells that can be stimulated to develop cartilage.[34] BMS is indicated for minor defects; for more significant focal defects (from 2 to 3 cm^2), the osteochondral autografts represent an attractive procedure. This procedure comprises the small plugs transferring bone and hyaline cartilage from the donor site to the defect. The donor locations are healthy joint regions with less weight bearing. For instance, this could be performed by transferring cylindrical osteocartilaginous plugs collected from the nonarticular area of the femur to the site of the defect.[35] In the arthroscopic debridement technique, the malfunctioning portions of cartilages and tissues are removed to help reduce pain and improve movement. This is used as an alternative to open debridement when the lesion is small, and it is possible to access the tendinosis tissue from the patellar tendon joint side.[36]

The surgical possibilities currently used have a limited capacity for tissue regeneration and yield only short-term relief symptoms. Consequently, IA joint injection approaches for VS, with a possibility of tissue regeneration, are rising. Specialists have usually used joint injection of HA as the nonoperative treatment of patients suffering from degenerative joint diseases. VS is an optional intervention to deal with OA symptoms and restore the joint through the IA injection of a polymer. In 1997, the first VS of Hyalgan (Fidia Pharma) was approved, an injectable formulation of the sodium salt of HA. HA is commonly found in the extracellular matrix of vertebrate epithelial, neural, and connective tissues. The clinical benefits of IA joint injection of HA are 1) the mechanical VS of the

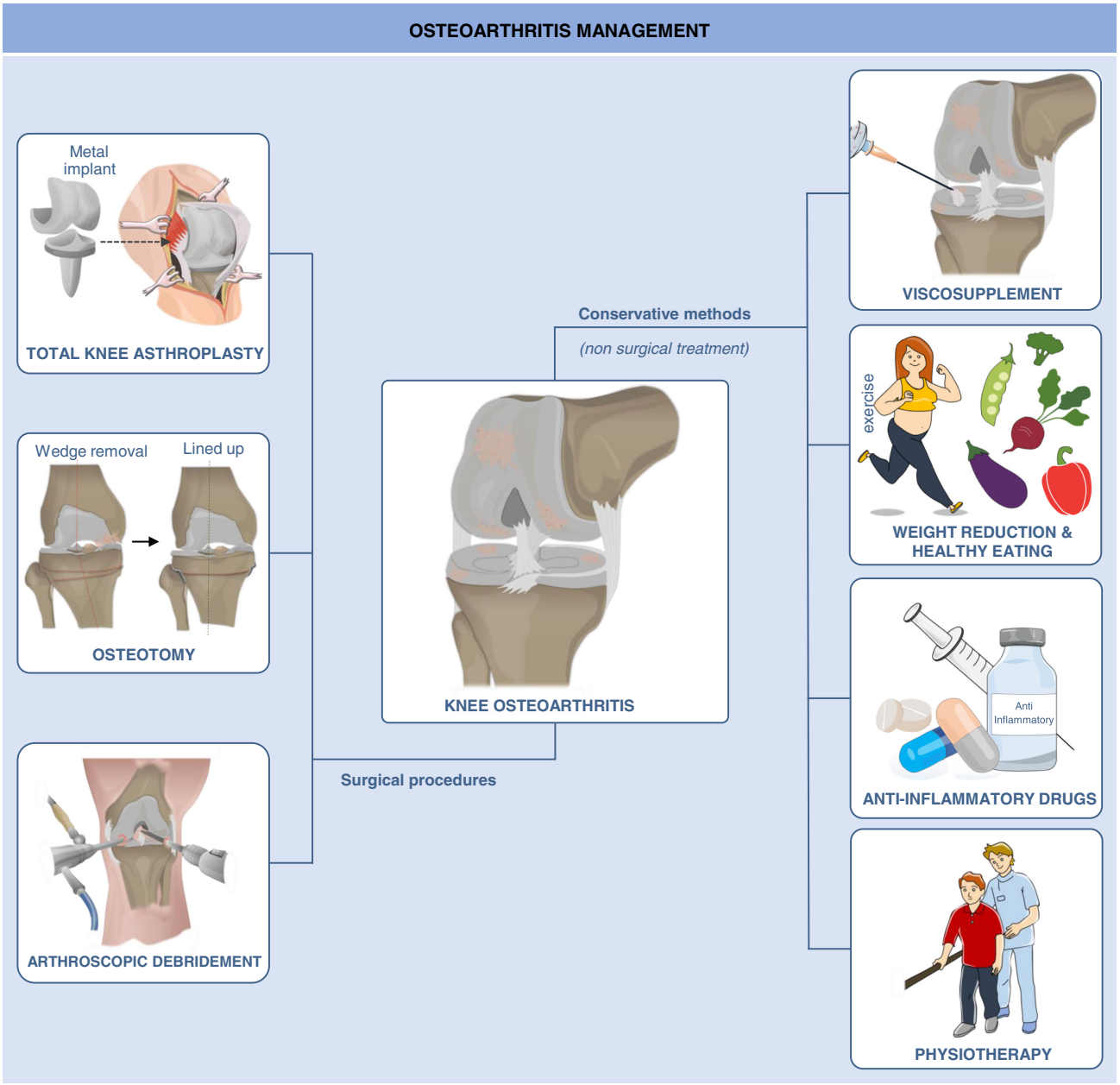

Figure 2. Current therapies for OA management.

joint, allowing lubrication and shock absorption, and 2) the re-establishment of joint homeostasis through the induction of endogenous. In fact, in OA conditions, the joint natural HA content has a less concentration, molecular weight, and a different structure. This adversely affects the synovial fluid rheological properties (lubrication, viscosity, and shock absorption).

HA is a glycosaminoglycan made from chains of disaccharide repeating units. These disaccharide units are formed from D-glucuronic acid and *N*-acetyl-d-glucosamine and are alternately repeated, establishing a chain-like structure with variable molecular weights. The HA present in the human synovial fluid has around 4000 kDa, with an exceptionally hydrophilic performance responsible for the lubricant properties. In addition, the joint synovial fluid is due to HA's extraordinary viscoelastic characteristics, critically relevant to the joint biomechanical functioning acting as a shock absorber and a lubricating layer.[37] The production and molecular weight of HA, and thus the quality of the synovial fluid, are lowered in an inflammatory state with OA progression. Moreover, HA produces antiarthritic effects via multiple mechanisms involving receptors, enzymes, and other metabolic pathways.

Engineering an immunomodulatory solution could also have an influence in resolving inflammation. High-molecular-weight HA creates a scarcer proinflammatory environment than

lower-MW HA.[38] Thus, HA molecular weight affects its immunomodulatory capacity. For instance, HA, over 1000 kDa, presents anti-inflammatory, filling, and hydrating functions, thus protecting β-cells from leukocyte-mediated death, while HA, ≈250 kDa, is highly angiogenic, immune stimulatory, and inflammatory (activating toll-like receptors).[39]

In the development of new viscosupplements, several properties are considered. Viscosupplements are different in their source, fabrication techniques, molecular weight, treatment schedule, and physicochemical properties. Usually, those mimicking the synovial fluid are the gold standard, but normal synovial fluid is present in a typical healthy joint. For example, it should be able to act as a space filler (it is naturally secreted into the joint and removed by the synovium), lubricant (protecting the cartilage surfaces from frictional damage), scavenger (the efficient scavenger of free radicals and cellular material), and also cellular activities' regulator (such as pain receptors). Therefore, HA IA injections can increase the synovial fluid flow, normalize the endogenous HA synthesis, inhibit its degradation, and reduce joint pain. Aside from its biomechanical performance, this polysaccharide also expresses a biological activity that ultimately retards disease symptoms (including pain) and delays the release of cartilage degradative enzymes by chondrocytes, like matrix metalloproteinases, recognized as important modulators of inflammatory response.

Moreover, the ideal viscosupplement product should not only seek the characteristics of normal synovial fluid but also go further with properties beneficial to recovering the damaged joint. Thus, it is of greater interest to develop a viscosupplement with properties such as being anti-inflammatory, an antioxidant, having immune modulation activities, adequate molecular weight, being biocompatible, noncytotoxic, and having improved resistance to hyaluronidase. More recently, the smart delivery of the drugs and biological components is being studied to improve the treatment effectiveness, resulting in the repair of the articular cartilage from the first to the last stage of OA progression.

The design and development of the VS should use a specific framework. First, design must fulfill particular outputs and go through a review procedure. Then, the development of the solutions begins. Then, when the product is set to be implemented, it must be evaluated against the specified readiness standards to ensure that it meets the established requirements throughout the validation process.

The design specifications of lubricity, cushioning, and residence time to specifically lubricate healthy and worn cartilage, provide cushioning, and reside in the joint after IA injection are there for more than 30 days. Research in new materials for VS is a field with many opportunities but also with many challenges. The experimental design arises as an important tool, which together with complete characterization will help the researchers to follow the best path to obtain the expected product. The design of experiments (DOE) conducts researches throughout process optimization at a reduced cost and time. It is a statistical and mathematical instrument to define the experimentations needed and systematically and efficiently assess the obtained results.[40] The known DOE weakness is linked to the range of the study that frankly affects the prediction. DOE could be divided into four major factions: 1) response surface methodology (RSM)-based DOE, 2) Latin square DOE, 3) Taguchi DOE, and 4) factorial DOE.[41] While Taguchi DOE is based on the prior choice of the most probable interactions, in standard fractional factorial designs, the interactions are selected afterward with the evaluation of the initial results. Rafidah and co-workers concluded that the full factorial design is better than the Taguchi method.[42]

The global VS market was valued at around USD 3.8 billion in 2019, and it is projected to achieve a value of almost USD 4.8 billion by the end of 2027. This market is exclusively dominated by HA and led primarily by aging but physically active population.[43] Hyalgan, Artzal, Suplazyn, BioHy, Orthovisc, Durolane (NASHA, nonanimal-stabilized HA, a new generation of common HA preparations with higher half life and high density), and Go-ON[43] are examples of formulations of HA for IA joint injection. A comparison between the rheological properties of available IA HA preparations (Euflexxa, Orthovisc, Supartz, Monovisc, Synvisc, Synvisc-One, Gel-One, and Hyalgan) and human synovial fluid concluded that Euflexxa is the most similar to healthy synovia regarding the molecular structure, shear rates, and crossover frequency of[44] HA production,[45] and it is generally safe and well tolerated. However, it also faces well-known limitations, such as the production cost and the need for repeated administration into the knee joint. In addition, it clears fast from the body due to the matrix metalloproteinase (MMP) degradation. Moreover, it is also predominantly inert when compared with other tissue engineering and regenerative medicine (TERM) approaches. These downsides open a wide window of opportunities to new engineered IA products for OA effective treatment.

For the past years, HA was the viscosupplement of choice. However, HA has a low residence time, presents a low-to-moderate clinical efficacy, and is not recommended in recent guidelines. Therefore, one may consider VS with other compounds or biomaterials. These new products need to have a longer residence time, a better clinical efficacy, and a disease-modifying effect.

Recently (in the past six years), the studies that focused on viscosupplement biomaterials for the treatment of OA are mainly related to: 1) slight changes to the formulations to 1) increase efficacy and 2) reduce the number of injections, together with 2) comparative studies of the existing solutions. For instance, Oliveira et al. (2018) studied the effects of the HA of different molecular weights in an experimental model of OA in rabbits from the immunohistochemical perspective.[46] Their results revealed that the saline solution demonstrated signs of OA development while adding native HA of low molecular weight (Hyalgan) and HA of high molecular weight (Synvisc) that protected the articular cartilage in this model of OA. Furthermore, an evaluation of the effectiveness and security of IA monoinjection of a novel crosslinked hyaluronan (HYA-JOINT Plus) and Synvisc-One was performed in patients with OA.[47] This study concluded that unique injection in patients with knee OA of both products gives similar safe and effective results (6 months). However, HYA-JOINT Plus is superior to Synvisc-One to reduce pain (VAS score) and stiffness (WOMAC: Index score of Western Ontario McMaster Universities Arthritis). Moreover, meta-analysis indicates that the single injection produces similar results to multi-injections (i.e., 3–5 injections) of IA HA in terms of pain relief (Western Ontario and MacMaster Universities pain

subscore) in the treatment of knee OA.[48] To explore the possibility of using single-injection HA to increase patient convenience while maintaining the therapeutic efficacy, Suppan et al. (2017) presented a trial performed under good clinical practice guidelines, using single 5 ml or the conventional three injections of 2.5 ml GO-ON at weekly intervals. The study showed good efficacy, tolerability, and safety of a single larger dose of GO-ON knee IA injection.[49] Other researchers found that HA injection and oral administration may have beneficial therapeutic effects on patients with early OA. However, different outcomes in younger and older subjects suggested combined therapy: 1) first with local infiltrations and 2) later with oral composition.[50] The clinical effects of HYADD 4 (HA with distinctive rheological properties) were evaluated in patients with mild-to-severe knee OA.[51] They found two consecutive infiltrations, 1 week apart, reduced WOMAC scores, and NSAIDs/acetaminophen consumption for at least 6 months. In a subpopulation, efficacy on pain lasted ≈12 months. However, adverse events were reported in 11.2% of patients; the most frequent was arthralgias. The study of the need to use other drugs after VS with Hylan G-F 20 was also explored. After 2 months of VS, patients with knee OA previously treated with opioids or IA CS injections exhibited a statistically significant decrease in medication.[52] Another study on Hylan G-F20 (HY) demonstrated that both protein-free saline-soluble galactomannan derived from guar gum (GM) and Hylan G-F20 (HY), besides exposing both analgesia and chondroprotection in experimental OA, yield mild synovitis with cytokine release after IA injection[53] (**Figure 3**). A study on the long-term survivorship after 5 years of IA injection of high-molecular-weight HA (GF-20, Synvisc-One) demonstrated a significantly longer duration of clinical benefit, representing an opportunity to delay the arthroplasty procedure.[54] The effectiveness of single-injection Hylan G-F20 was also attested in another study, being well tolerated with very few patients experiencing any treatment-related adverse events.[55]

Studies have been done regarding the use of oxygen–ozone injections on OA knee.[56,57] The reduction of pain, joint functional improvement, and quality of life of those injections, when compared with placebo, were studied by Jesus et al.[56] These authors confirmed the efficacy of this approach after 8 weeks of treatment in 98 patients with symptomatic knee OA divided into two groups: 1) IA 20 μg ml^{-1} of ozone or 2) placebo. Another work studied the comparison between IA injection of HA, oxygen–ozone, and their combination in the knee OA treatment. The results showed that applying O_2O_3 and HA led to a significantly better outcome than HA and O_2O_3, given separately to patients affected by OA of the knee.[58]

A placebo-controlled research assessing the efficacy of IA injections of HA and a novel HA-platelet-rich plasma (PRP) conjugate in a canine model of OA was recently studied,[59] as shown in **Figure 4**. These authors found that both injections of HA and HA-PRP may be enough for short-range improvement of OA symptoms. Still, treatment with HA-PRP significantly offers better long-term cartilage preservation. Patients treated either by autologous leukocyte-rich L-PRP or HA IA knee injections, administered in series of three at 1-week intervals, were analyzed in contrast with the indication described by in vitro studies, where a cellular proinflammatory response appears to be induced by the presence of leukocytes; these results suggest that leukocyte-rich PRP does not induce pertinent in vivo upregulation of proinflammatory mediators.[60] Chen et al. (2016) proposed a treatment strategy of PRP in association with HA injection to treat severe knee OA, rather than immediate surgery, or an opportunity for those who cannot go through surgery. It can also postpone arthroplasty and can significantly improve the daily activity function.[61] Moreover, PRP is significantly more effective at 1 year due to an incremental cost-effectiveness ratio than HA PRP injections that could be thought of as a realistic and appropriate option for HA injections for symptomatic knee OA treatment.[62] Comparing PRP versus HA viscosupplements in terms of symptoms' relief and time for arthroplasty revealed that the first is associated with better outcomes and might prolong the time for arthroplasty and provide an effective therapeutic option in selected patients with knee OA not responding to conventional

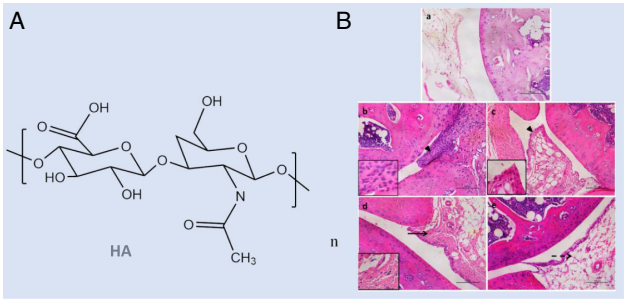

Figure 3. Main composition of the viscosupplement, A) HA and B) histological images: a) saline-injected joints appearing normal; b) mild synovial hyperplasia (arrowheads) in GM, and c) HY-injected joints at 7 days; d) at 28 days, no long synovial hyperplasia with mild fibrosis in HY (arrow; inset); e) seemingly typical fat subsynovial tissue in a GM-injected joint (dashed arrow) (original magnification: 200×). Reproduced under a Creative Commons Attribution (CC BY 4.0) license.[53] Copyright 2020, The Authors. Published by Springer Nature.

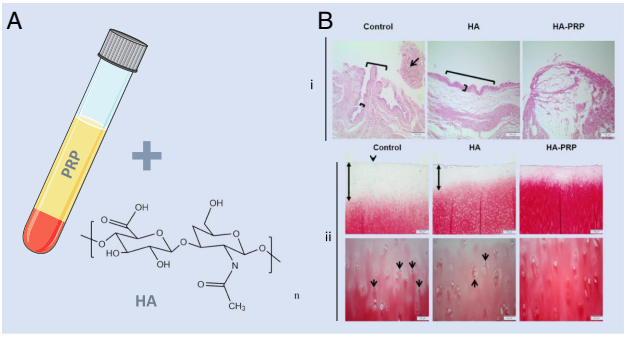

Figure 4. A) The main compositions of the viscosupplement, PRP and HA, and B) histological images, in which (a) is synovium and (b) the cartilage; cell layer (small arch) and villous hyperplasia (long arch) detected in the control and HA groups' synovium. Mild-to-moderate inflammatory cell infiltrates were identified in the control group. The articular cartilage at the superficial zone with an irregular surface (arrowhead), control group. The grade of proteoglycan staining (bidirectional arrows) was the lowest in the control group, followed by the HA group. The larger cell clusters in chondrocyte (short-body arrows) were observed frequently compared with the HA and HA-PRP group. Reproduced under a Creative Commons Attribution (CC BY 4.0) license.[59] Copyright 2019, The Authors. Published by Springer Nature.

treatments.[63] The combined use of PRP and HA is also considered. It is a safe and efficient procedure that provides functional benefits and is significantly better than HA injective therapy alone.[64] Moreover, athletes with chronic degenerative cartilage lesions of the knee responded positively both to HA and to PRP.[65] Evaluating the rheological and biological properties of different HA compositions in combination with PRP revealed that PRP addition is not detrimental to the VS effect of HA, as the viscoelastic properties are lost.[66] Huang et al. (2019) explored whether IA injections of PRP are superior to HA or CSs for early stages of knee OA, indicating that IA-PRP showed significantly lower scores long term.[67]

Chondroitin sulfate (CS) is an important element of bone and cartilage, being extensively investigated for OA treatment. The evaluation of the pharmacokinetic (PK) properties of both HA and CS after IA administration in a validated OA animal model was performed. Despite differences in their molecular size, both exhibited PK behavior equally characterized by prolonged residence within the joint and slow release in plasma, favoring long-term beneficial effects.[68] In addition, a study aiming to evaluate the safety and performance of the IA VS agent HYALGO, a formulation combining HA (>1700 kDa) and CS, in the treatment of patients suffering from knee OA, was performed. This study concluded that three injections of HYALGO were safe and effective to manage symptomatic knee OA, with a beneficial effect that increased progressively over time, peaking 6 months after injection.[69] The histological changes in knee cartilage and bone following the administration of two different CS products in two experimental OA models (MIA) in rats were studied (**Figure 5**). It was found that high-quality pharmaceutical-grade CS ensures optimal efficacy and safety of the final product in patients with OA.[70] CS combined with HA yielded some commercially available VS, such as Arthrum HCS and Synovium surgical.

Studying the safety and efficacy of a VS made of intermediate-molecular-weight HA mixed with a high concentration of mannitol (HAnOX-M) with a marketed high-MW HA (Bio-HA) in patients with knee OA revealed that HAnOX-M has similar safety and effectiveness to Bio-HA.[71] The treatment with

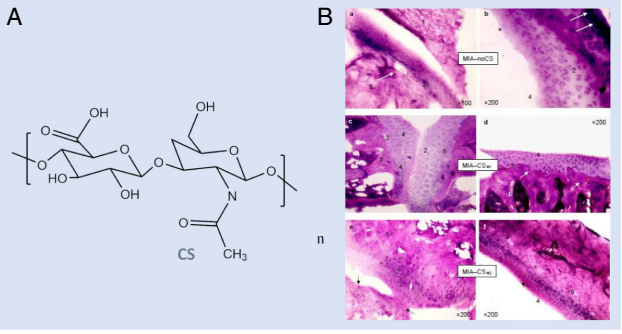

Figure 5. Main composition of the viscosupplement, A) CS and B) histological images, in which the articular cartilage of the knee joints of rats in a,b) MIA–noCS, c,d) MIA–CS #1, and e,f) MIA–CS #2 groups is seen. MIA–noCS rats with mono-iodoacetate-induced OA give no CS. Arrows show a,b) bone destruction, d) neovasculogenesis, e) deformed contour, and f) active proliferation. Adapted under a Creative Commons Attribution (CC-BY 4.0) license.[70] Copyright 2021, The Authors. Published by EJCEM.

one injection of the crosslinked high-molecular-weight HA combined with mannitol in patients with knee OA effectively alleviated symptoms over 6 months, without safety concerns.[72] In addition, the effect of IA injection of a reticulated HA with mannitol (KARTILAGE CROSS) on the level of a specific biomarker of collagen type-II degradation was assessed. A beneficial impact on cartilage degradation was discovered.[73]

Rieger et al. (2017) studied the IA injection of a hybrid hydrogel (chitosan added to HA) in a rabbit model of early OA. Comparing results from three groups (treated with IA injections of saline, HA, and hybrid compounds), the hybrid hydrogel improved the microarchitectural parameters and mineral density changes.[74] An alternative delivery system was studied based on HA hydrogel and poly(lactic-*co*-glycolic acid) (PLGA) particles with oleic acid. The in vitro HA release from PLGA particles revealed a sustained profile. Particles showed good in vitro cell compatibility with no risk of hemolysis (less <1%), and the in vivo anti-inflammatory study showed a higher inhibition for HA-loaded PLGA particles when compared with HA solution (78% vs 60%), suggesting that this formulation may be a promising alternative to HA commercial dosage form.[75]

Pashuck et al. (2016) studied the security and efficiency of IA injections of HA versus saline for symptomatic treatment of OA in a canine model. They discovered that the three HA injection protocols were safe, superior to saline for short-term amelioration of symptoms associated with chronic OA, and can be translated to human OA treatment.[76] To assess the clinical results of IA infiltration with HA and dexamethasone alone and in combination with the treatment of knee OA, Maia et al.[77] evaluated 44 patients who underwent treatment for OA. According to the WOMAC total score and subscores, the authors reported that VS isolated enhanced pain, stiffness, and function and improved knee extensor and flexor strength, but not proprioception until six months after infiltration, suggesting that VS has a positive outcome on quadriceps arthrogenic inhibition.

35 patients with knee OA and 50 asymptomatic subjects were evaluated 1 day, 2, and 4 weeks after injection of the 1.5% crosslinked HA to assess the influence of VS on osteoarthritic knee arthrokinematics. This was performed by vibroacoustic emission, smooth movement in the joint, to verify the friction-reducing properties of the articular environment. They found that VS may be helpful for arthrokinematics recovery in quite short periods.[78] Another study on the effects of VS on knee joint arthrokinematics similarly showed that joint motion-related vibrations were reduced after IA-HA injection.[79] In addition, Telikicherla and Kamath[77] performed distinctive work to understand the correct needle placement inside the knee joint before VS. It showed that the accuracy was higher through the lateral midpatellar than the anterolateral portal.

Currently, under investigation or in the clinical trial phase, some other products such as CSs and PRP are used as viscosupplement products. Even so, there still do not exist any significant and direct comparative studies between the IA products based on HA, PRP, and CSs that prove the best products and their effectiveness to be used as injectable materials for the treatment of OA.[67] In the case of CSs, it is widely recommended to be used during the inflammatory phase of OA due to its efficiency to affect the T and B cell functions, cytokines, and enzymes, resulting in a substantial reduction of tissue

inflammation, reported in several recent studies of their efficiency in providing significant pain relief at short term (from 1 week up to 4 weeks).[80] However, PRP is considered a current therapy that uses local injections of a higher concentration of the patient's own platelets to quicken the healing of this damaged tissue. However, this type of therapy only aims to reduce pain and improve the natural physical tissue functions and has the advantage of being from an autologous nature and the lack of side effects typical of other common on-the-shelf pharmaceuticals.[81] Unfortunately, despite all current advances, there is no cure already found to treat the OA disease, being those in practice almost palliative approaches. This demands an effort to develop more innovative products solo or combined, as described in **Table 1** in the preclinical status.[80–94]

In the past 5 years, many products have been described in the literature (Table 1). Still, as seen later (**Table 2**), few have reached the preclinical and clinical stages, possibly verifying the total dominance of HA formulations. The HA formulations approved for knee OA are classified as crosslinked or non-crosslinked, further defined by chemical modifications and production methods. For instance, Hylan G-F20 is chemically modified (crosslinked HA) to increase the molecular weight, aiming to replicate the

Table 1. Viscosupplement biomaterials for treatment of OA according to current literature.

Formulations	Study title	Publish data	Outcome	Refs.
Hyaluronate versus hyaluronate with CS and *N*-acetyl-D-glucosamine	Comparison of the chondroprotective effect of a novel hydrogel compound and traditional hyaluronate on rat cartilage in apapain-induced OA mode	August 2016	The combined formulation is more chondroprotective to rats cartilage during the early stage of OA. (Mw HA: 1200 kDa)	[82]
HA	Comparison of two different-molecular-weight IA injections of HA for the treatment of knee OA	March 2016	Low- or high-molecular-weight HA improves stiffness, joint function, and pain. However, no clear benefit was observed between this two. (Mw: 1550 kDa)	[83]
	Interaction with cartilage increases the viscosity of HA solutions	May 2020	The effective viscosity of HA demonstrates to be more relevant to lubrication than the bulk viscosity traditionally assessed in cartilage surface. (Different Mw: between 300 and 1800 kDa)	[84]
	Tribological effectiveness of viscosupplements for OA in knee joint	September 2019	The results show that a high concentration of viscosupplement is more effective for the treatment of OA. (no specific Mw)	[85]
	Rheological study of HA derivatives	February 2017	These compounds demonstrate that the HA solutions are not only dependent on polymer concentration, average molecular weight, and degree of crosslinking but may also be affected by the method of deacetylation and butyrylation. (Mw: 30–214 kDa)	[86]
Polynucleotides (Condrotide)	IA treatment of osteoarthropathy knee with polynucleotides: a pilot study with medium-term follow-up	June 2013	The results demonstrate that the administration of nucleotides in subjects with both severe knee arthritis and chondropathy can be recommended. (no specific Mw)	[87]
Lactose-modified chitosan–hydrochloride from comparing with HA	A hydrogel system based on lactose-modified chitosan for VS in OA	July 2020	The results support the use of this hydrogel biomaterial in a pathological condition involving inflammation and provide evidences for the potential beneficial effects for OA. (Mw HA between: 1500–1800 kDa)	[88]
Hyaluronan sodium salt with gellan gun and oleuropein. Crosslinked with calcium ions	Calcium ions hyaluronan/ gellan gum protective shell for delivery of oleuropein in the knee	November 2020	The biomaterial creates a protective shell for the delivery of pharm oleuropein. The results show that this system permits the pharm's release and can act as an anti-inflammatory with analgesic effects. (Mw HA: 2000 kDa)	[89]
HA versus hymovis®	Inflammatory and noninflammatory synovial fluids exhibit new and distinct tribological endotypes	November 2020	Investigate the effects of the HA VS Hymovis, with the addition of HA significantly increasing all fluids' viscosities. (no specific Mw)	[90]
Hylan G-F 20 versus opioid prescriptions	Evaluation of Hylan G-F 20 treatment with opioid prescriptions and IA CS injections in patients with osteoarthrosis of the knee using a claims database	October 2020	Hylan G-F 20 is linked to a decrease in opioid recommendations. (Mw Hylan G-F: 6000 kDa)	[91]

Table 1. Continued.

Formulations	Study title	Publish data	Outcome	Refs.
HA conjugated with purified (RegenoGel) or autologous plasma-derived fibrinogen (RegenoGel-OSP)	A novel approach for knee OA using high-molecular-weight HA conjugated with plasma fibrinogen: interim findings of a double-blind clinical study	July 2020	RegenoGel and RegenoGel-OSP are secure and potentially efficient in alleviating pain and symptoms of knee OA, for at least 6 months (Mw HA: 1600 kDa)	[92]
Poly (7-oxanorbornene-2-carboxylate) polymer with triethylene glycol (TEG)	A synthetic bottle brush polyelectrolyte reduces friction and wear of intact and previously worn cartilage	June 2019	The results demonstrate good lubrication properties in the treatment of early-stage OA in ex vivo worn cartilage. (Mw: 2000 kDa)	[93]
Poly-oxanorbornane carboxylate	A synthetic polymeric biolubricant imparts chondroprotection in a rat meniscal tear model	November 2018	This synthetic biolubricant provides longer-lasting and more effective lubrication than a current viscosupplement used in ex vivo. (Mw: 2400 kDa)	[94]
Mannitol-modified crosslinked HA	Safety and predictive factors of short-term efficacy of a single injection of mannitol-modified crosslinked HA in patients with trapeziometacarpalOA. Results of a multicenter prospective Open-Label Pilot Study (INSTINCT Trial)	June 2021	This study suggests that a single injection is effective in relieving pain in patients with OA. (no specific Mw)	[108]
Dendritic polyglycerol sulfate	Injectable hydrogels for treatment of OA – A rheological study	November 2017	These hydrogels have the potential to be an attractive alternative for HA as an IA injectable material (no specific Mw)	[109]

Table 2. Viscosupplement biomaterials for the treatment of OA currently used in the clinical trial.

Formulations[a)]	Study title	Clinical status and last update	Outcome	Reference or Identifier
HA (hyaluronan)	Comparing one IA injection of a novel HYAJOINT plus with synvisc-one for the treatment of knee OA.	Completed February 19, 2016	*(NA.)* (Mw is not specified)	NCT 02686047
	A study on visco-antalgic IA administration in symptomatic knee OA	Completed June 11, 2020	*(NA.)* (Mw is not specified)	NCT 02740231
	Nonanimal HA for the treatment of ankle OA: A prospective, single-arm cohort study	(Published article) 2018	VS with nonanimal HA are promising and a single injection was associated with clinically meaningful reductions in pain and disability for up to 26 weeks. (Mw is not specified)	[100]
	Stem cell and growth factor injury and arthritis clinical research study	Completed March 25, 2021	*(NA.)*	NCT 03408145
	Clinical comparison of oral administration and VS of HA in early knee OA	(Published article) 2017	Results show that HA injection and oral administration may have beneficial therapeutic effects on patients with early OA. (Mw is not specified)	[101]
	Pilot, prospective, comparative multicentric study evaluating the effects on the arthrosis biomarkers, the clinical effectiveness, the tolerance of an IA injection of HA kartilage cross versus placebo in patients suffering from knee OA	Completed November 1, 2016	*(NA.)*	NCT 02951585
	The safety and efficacy of single IA-HA injection in patients with kneeOA: A prospective study	Completed October 14, 2020	*(NA.)*	NCT 04577521

Table 2. Continued.

Formulations[a)]	Study title	Clinical status and last update	Outcome	Reference or Identifier
	A single IAinjection of Gel-200 for Treatment of symptomatic OA of the knee is more effective than phosphate-buffered saline at 6 months: A subgroup analysis of a multicenter, randomized controlled trial	(Published article) 2019	These treatment benefits patients with knee OA and shorter the disease duration, including for those at an early OA symptom stage. (Mw is not specified)	[102]
	The efficacy of VS for early knee OA	Unknown June 18, 2012	*(NA.)*	NCT 01210742
	Injection site diversity influences HA distribution and clinical results in CP and KOA	Completed July 26, 2018	*(NA.)*	NCT 03600571
	Early VS after anterior cruciate ligament reconstruction: A randomized controlled trial	(Published article) 2016	A single injection of HA, after 1 day after anterior cruciate ligament reconstruction did not produce adverse events. (Mw is not specified)	[103]
	Efficacy of a single IA injection of ultrahigh-molecular weight HA for hip OA: a randomized controlled study	(Published article) 2017	The results show that a single dose of HA is safe and effective. Furthermore, no significant difference in the clinical outcomes was found between ultrahigh- and medium-molecular-weight HA and a single dose is as effective. (Mw: 1300-3600 kDa)	[104]
	Efficacy of three weekly injections of a bacterial-sourced hyaluronate on pain and function in patients with knee OA	Completed August 20, 2010	*(NA.)* (Mw: 500–1200 kDa)	NCT 01185444
	Efficacy and safety of IA sodium hyaluronate single injection in patients with OA of the knee	Unknown March 1, 2019	*(NA.)*	NCT 03852914
Hyaluronan (HYA-JOINT Plus) with Synvisc-One	Comparison of single IA injection of novel hyaluronan (HYA-JOINT Plus) with Synvisc-One for kneeOA: A randomized, controlled, double-blind trial of efficacy and safety	(Published article) 2017	HYA-JOINT Plus or Synvisc-One is effective and safe for the treatment of KOA over 6 months. However, HYA-JOINT Plus is superior to Synvisc-One in terms of reduction of pain. (Mw is not specified)	[105]
PRP	PRP versus VS for the treatment of early knee articular degenerative pathology	Completed August 8, 2018	*(NA.)* (Mw HA: 1500 kDa)	NCT 02135367
	PRP versus VS in the treatment of knee articular degenerative pathology	Completed October 30, 2017	*(NA.)* (Mw HA: 1500 kDa)	NCT 01670578
HA and/or PRP	Knee OA: PRP or HA	Enrolling by invitation July 8. 2019	*(NA.)* (Mw: 800–1200 kDa)	NCT 03801564
	IA PRP compared with VS in the treatment of knee OA	Recruiting August 22, 2019	*(NA.)*	NCT 03491761
	IA injections of PRP versus HA in patients with kneeOA: Preliminary follow-up results at 6 months	(Published article) 2021	The preliminary results indicate that although the PRP injection can significantly improve clinical outcomes, it is not more effective than HA and is associated with higher costs and treatment times. (Mw is not specified)	[106]
	PRP versus HA injections for the treatment of knee OA	(Published article) 2018	Overall, PRP did not offer superior clinical improvement, in contrast with HA. (Mw HA: >1500 kDa)	[81]
HA, PRP, and CS	IA injections of platelet-rich plasma, HA, or CSs for kneeOA: A prospective randomized controlled study	(Published article) 2019	Observed is a significant reduction in pain and clinical improvement after 3 months. (Mw HA: 500–730 kDa)	[67]
HA versus botulin toxin	A trial comparing botulin toxin versus HA by IA injection for the treatment of painful knee OA	Recruiting August 25, 2020	*(NA.)*	NCT 02832713

Table 2. Continued.

Formulations[a)]	Study title	Clinical status and last update	Outcome	Reference or Identifier
HA versus CS	A study comparing VS and CS injections for knee OA	Unknown May 28, 2010	*(NA.)*	NCT 01132677
HA with mannitol	Safety and efficacy of IA injections of a combination of HA and mannitol (HAnOX-M) in patients with symptomatic kneeOA: Results of a double-blind, controlled, multicenter, randomized trial	(Published article) 2016	This product is an effective and well-tolerated treatment for knee OA, which allows long-lasting pain relief, decreased analgesic consumption, and functional improvement. (Mw: 1500 kDa)	[110]
	Reduction of the serum levels of a specific biomarker of cartilage degradation (Coll2-1) by HA (KARTILAGE CROSS) compared with placebo in painful knee OA patients: the EPIKART study, a pilot prospective comparative randomized, double-blind trial	(Published article) 2017	The results demonstrated that VS significantly decreased serum Coll2-1, a maker of cartilage catabolism, compared with the injection of a saline solution. Suggesting that could be used for the assessment of a single IA treatment in clinical trials (Mw is not specified)	[111]
HA with triamcinolone hexacetonide	Comparison of two application regimens for VS	Completed January 6, 2015	*(NA.)* (Mw is not specified)	NCT 01824485
HA with/out dexamethasone	VS improves pain, function, and muscle strength, but not proprioception, in patients with kneeOA: a prospective randomized trial	(Published article) 2019	The treatment with both materials was more effective than the VS alone, which improves pain, stiffness, and function. (Mw is not specified)	[112]
HA and polynucleotides	Comparative assessment of VS with polynucleotides and HA	Completed December 26, 2017	*(NA.)* (Mw is not specified)	NCT 02417610
Chitosan	Safety performance chitosan OA VS	Unknown September 21, 2018	*(NA.)* (Mw is not specified)	NCT 03679208
Plasma rich in growth factors	Effect of plasma rich in growth factors in knee OA	Completed May 5, 2015	*(NA.)*	NCT 02039531
CS (ENKO 1) and durolane	Efficacy and safety of an IA injection, ENKO 1, in patients with symptomatic knee OA	Completed March 23, 2021	*(NA.)*	CT 03762408
Collagen-PVP and Hylan G-F 20	Collagen-PVP versus Hylan G-F 20 in the treatment of knee OA	Recruiting July 17, 2019	*(NA.)*	NCT 04019782
Hylan G-F 20	Pilot study of therapy with Hylan G-F 20 exercise capacity	Terminated March 29, 2016	*(NA.)*	NCT 01810848
Hylan G-F 20, ketorolac tromethamine, and methylprednisolone acetate	Knee injection RCT	Terminated February 18, 2020	*(NA.)*	NCT 03694821
Triamcinolone and/or Hylan G-F 20	VS in patients with severe OA of the knee: 6 follow-up of a randomized, double-blind clinical trial	(Published article) 2017	The treatment improves the treatment of inpatients with severe OA of the knee. However, such improvement was not superior to that obtained with IA triamcinolone. (Mw is not specified)	[113]
Hylan G-F 20 and triamcinolone	Evaluation of the effect of adding CS to VS	Completed December 23, 2014	*(NA.)*	NCT 01335321

[a)] *(N.A.)* = not available data.

properties of native synovial fluid more closely and lengthen IA residence half life. However, there is no conclusive evidence that differences in viscosupplement physical properties translate into superior clinical efficacy.[95] The properties sought are mainly the molecule network, rheological properties, water homeostasis, network interactions with other macromolecules, and matrix and cell surface interactions.

From Table 1, a wide range of molecular weights for HA-based products (1600–6000 kDa) are used; the other products do not even mention it. The molecular weight affects several other

properties, such as the rheological and network interactions. Regarding the molecular structure, the molecules recently under consideration, besides HA, are very distinct, indicating that a specific chemical structure (similar to HA, for instance) is not an essential factor or a prerequisite to achieve a successful product.

3. Translation to the Clinic

Despite the existence of chemical, pharmacological drugs, and some natural products (e.g., HA, chitosan, among others) with similar properties (e.g., anti-inflammatory properties), some international standard organizations such as the European League Against Rheumatism (EULAR), American College of Rheumatology (ACR), American Academy of Orthopedic Surgeons (AAOS), OARSI, and the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis, and Musculoskeletal Diseases (ESCEO) recommend the use of nonpharmacologic treatments as first line, and also adopt a healthy life such as losing weight, having good nutrition, and exercise.[96] However, in situations where the OA disease is very advanced and severe, the best alternative to avoid surgical procedures is viscosupplement products.

In recent viscosupplement studies, HA is the most investigated for OA treatment due to its natural properties to 1) restore joint lubrification (synovial fluid); 2) absorb shock; 3) reduce the inflammation process, and 4) reduce the mechanical stress of the cartilage tissue.[97] However, this lack may be surpassed in future research, exploring new and innovative products. Furthermore, these products should be intrinsically bioactive (to reduce inflammation and pain) and be effective in (compared with the products already explored) to treat this disease long term. Overall, the most preclinical conclusive studies demonstrate that most viscosupplements studies can provide good lubrification and reduce the symptoms of knee OA in the early and late stages. However, to fully validate the effectiveness of these innovative products, clinical trials are required.

Clinically, recent evidence suggested that the viscosupplements based in HA, CS, and PRP significantly improve pain and natural physical functions.[67,83] However, in addition to preclinical status, comparative studies and their efficacy are pretty limited, unclear, and controversial. Furthermore, some dissimilar studies include the severity of OA disease, joint locations (e.g., knee, ankle, shoulders), number of injections needed, treatment time, and the molecular weight of the products, among others.[98] Nevertheless, despite the considerable interest in these products, many studies in the clinical stage also combine this with other drugs such as dexamethasone, hexacetonide, mannitol, and botulin toxin, among others, as well as the use of other innovative products that are described in Table 2.[96–106]

When a team takes their product into clinical trials, there are unavoidable safety concerns and issues to consider. These are first 1) transversal to all clinical products and then 2) those associated with the product itself have to be weighed. For instance, the main potential concerns about pursuing newly discovered drugs or devices for preclinical and clinical trials are related to the possibility of side effects, not working, or the participants giving up. Moreover, those safety concerns related to the product itself had to be previously proven to be safe in in vitro and in vivo (animal) testing. Even so, risks associated with the product exist; they could be related to the patient tolerability to the dosage and how it is administered and specific biological responses. For instance, HA-based products have outstanding safety and a tolerability profile with few severe side effects. Side effects are usually local, with transient pain and swelling at the injection site, and include a greater frequency of joint pain and swelling than placebo.[99]

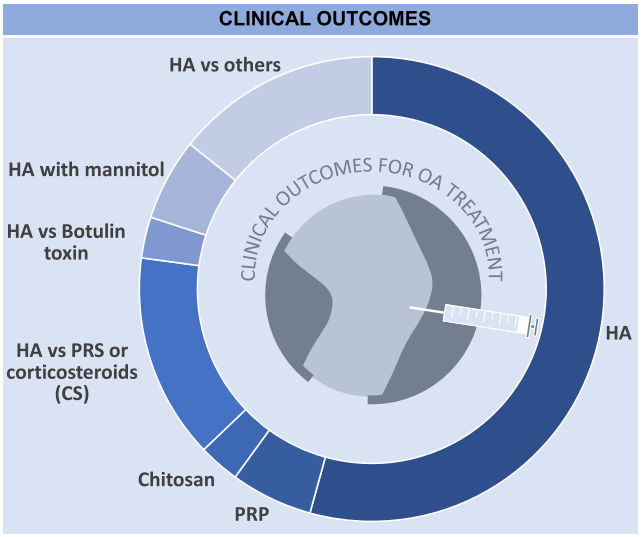

Figure 6. Schematic summary of current clinical outcomes of viscosupplement biomaterials for the treatment of OA.

HA remains the most used product for OA treatment in clinical trials, followed by PRP and CS. However, despite the advantages of these products (e.g., an immediate effect on reducing pain), there is much evidence that high-molecular-weight HA can provide better results in improving tissue functions at mid and long term compared with PRP and CS.[80,107] Unfortunately, after comparing all these data, there is no role for the time required for the treatment of this disease, being dependent on multivariables that make impossible the complete treatment in the short term (no less than 3 months). **Figure 6** summarizes the clinical outcomes of viscosupplement biomaterials for the treatment of OA.

A better quality of life is anticipated for OA patients, considering the work being developed at the moment. Forthcoming approaches can be envisioned with exceptional and compelling products: being able to restore the damaged tissue, being effective in the short term and with fewer injections, and using less-invasive and painful treatment procedures.

4. Conclusion and Perspectives

Nowadays, the treatments that use VS as a procedure are extremely valuable to avoid surgical practices predominantly in the early stages of OA as there is no cure for this disease. Currently, the most common viscosupplement products used in both scientific studies (preclinical and clinical trials) are HA, PRP, and CSs. These viscosupplements can be used as

isolated products or by being combined themselves and/or with other products such as pharmaceutical anti-inflammatory drugs. In general, even though all products discussed in this review have excellent properties such as being effective in restoring the natural function of the cartilaginous tissue, HA is the most used viscosupplement, as it can provide superior clinical improvements compared with other products, reducing in this way the OA symptoms like inflammation in the short term and being less painful for patients. Thus, the area of VS has auspicious resources to meaningfully contribute to restore the quality of life of OA patients. Furthermore, future experiments should expand such treatments to another level using biomaterials from natural and renewal resources with intrinsic bioactivity and rheology that can be scaled up and be available at competitive prices for orthopedic purposes.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

hyaluronic acid, intra-articular injections, knee osteoarthritis, platelet-rich plasma

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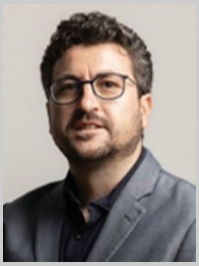

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