Mastelli
At the origin of regeneration

TRAINING MANUAL CONDROTIDE-CONROTIDEHA

PURPOSE AND STRUCTURE OF THE TRAINING

MANUAL

This manual is aimed at training medical sales representatives, introducing the osteoarticular pathology and therapy with a final focus on Condrotide HA® product.

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CHAPTER 1

ANATOMY AND PHYSIOLOGY OF THE JOINTS

Normal knee joint

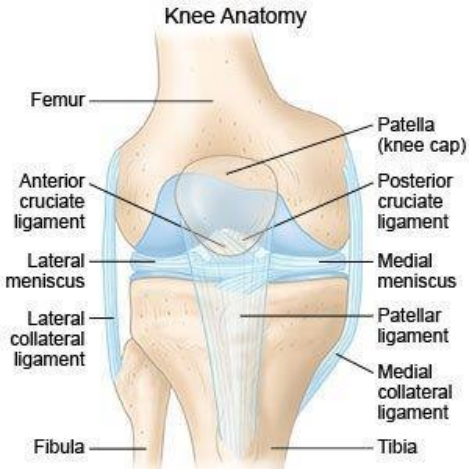


Image source: Drugs.com

Subchondral bone - The part of the bone located just under the cartilage is called the "subchondral bone". It is "the foundation" of cartilage.

The cartilage - Cartilage is a tissue that forms the lining of the bone ends. It has a pearlescent appearance and its thickness varies from one joint to another. Thus it is the kneecap that has the thickest cartilage: more than 5 millimeters. It is also worth knowing that men's cartilage is generally thicker than that of women[1].

The role of cartilage is to allow **perfect sliding between the bones** since the frictional forces are less than that of an ice skater sliding on ice. It is also essential for cushioning and distributing pressure on the bones thanks to its properties of elasticity and resistance.

Cartilage is a living tissue, in perpetual renewal, even among older people: it renews itself completely around every 3 months. Hence, microscopic cartilage fragments constantly break away from the cartilage and end up in the joint cavity (where they are then eliminated by the synovial membrane).

The joint fluid

Joint fluid also called synovial fluid is secreted by the synovial membrane. It is present in all joints in small quantities (1 to 2 ml in the knee for example). Its role is to **lubricate the joint.** It ensures perfect sliding between the bone ends.

In its normal state it is a clear, **transparent fluid with a particular viscosity**. The latter is **due to the amount of hyaluronic acid present in the joint.**

The capsule and the synovial membrane

The capsule and synovial membrane are closely linked since the synovial membrane coats the inside of the capsule, a fibrous covering surrounding the joint.

The synovial membrane is a vascularized and innervated tissue. It is in the form of a smooth transparent membrane covered with small blood vessels. In its normal state it has folds called villi.

Its main role is to **secrete the hyaluronic acid of the synovial fluid,** a true lubricant of the joint. The synovial membrane also ensures "emptying" of cartilage debris found in the joint cavity. This is thanks to its blood vessels that bring oxygen and nutrients, including glucose, essential for the life of the cartilage.

At microscopic level, the synovial membrane is composed of cells called synoviocytes. It is these cells that perform the functions mentioned above. Lastly, these cells also have the ability to produce enzymes that can destroy the cartilage matrix[2,3].

CHAPTER II

OSTEOARTHROSIS (OA)

2.1 Definition

Osteoarthrosis is a degenerative joint disease, in which the tissues in the joint break down over time. It is more common in older people.

People with Osteoarthrosis usually have joint pain and, after rest or inactivity, stiffness for a short period of time. The most commonly affected joints include the:

Hands (ends of the fingers and at the base and ends of the thumbs).

Knees

Hips

Neck

Lower back

Osteoarthrosis affects each person differently. For some people, Osteoarthrosis is relatively mild and does not affect day-to-day activities. For others, it causes significant pain and disability. Joint damage usually develops gradually over years, although it could worsen quickly in some people[4].

Researchers do not know what triggers or starts the breakdown of the tissues in the joint. However, as osteoarthritis begins to develop, it can damage all the areas of the joint, including:

- Cartilage, the tissue that covers the ends where two bones meet to form a joint.
- Tendons and ligaments.
- Synovium, the lining of the joint.
- Bone.
- Meniscus in the knee.

2.2 Epidemiology and burden of disease

Osteoarthrosis (OA) is the most common form of arthritis, affecting millions of people worldwide. It occurs when the protective cartilage that cushions the ends of the bones wears down over time. In Italy this chronic disease affects about 8 million people[5]. In Latin America, Mexico is in first position as for OA publications.

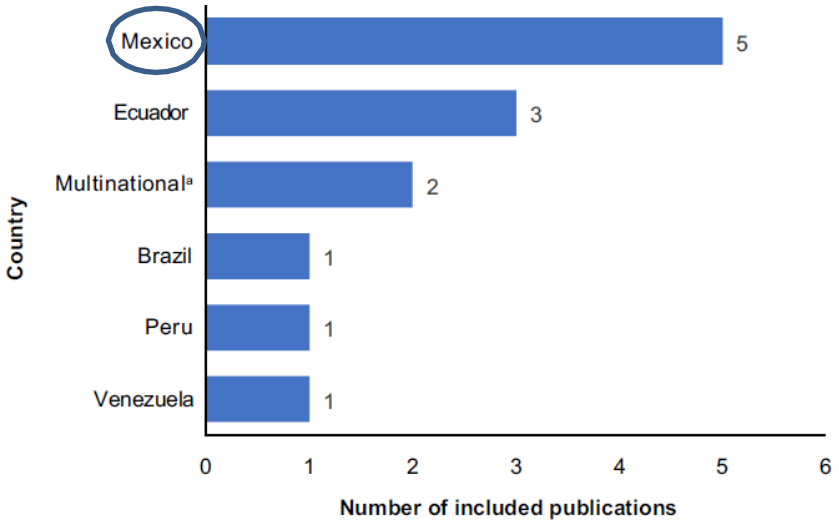


Table 2 Overall prevalence of symptomatic knee OA in 5 publications

First author, year/design	Registry, country	Population size, *n*			Population	Prevalence of knee OA, %		
		Total	Men	Women		Total	Men	Women
Granados, 2015 [12] cross-sectional	Venezuela	3973	1606	2367	Men and women ≥ 18 years of age	5.4	NR	NR
Guevara, 2019 [13] cross-sectional	Ecuador	2687	997	1690	Men and women ≥ 18 years of age	6.5	4.5	7.8*
Guevara-Pacheco, 2016 [14] cross-sectional	Ecuador	4877	1961	2916	Men and women ≥ 18 years of age	7.4	4.5	9.3**
Macias-Hernandez, 2018 [18] cross-sectional	Mexico	204	80	124	Men and women ≥ 40 years of age	19.6	12.5	24.2***
					Men and women 40–49 years of age	6.4	NR	NR
					Men and women 50–59 years of age	7.4	NR	NR
					Men and women 60–79 years of age	4.9	NR	NR
					Men and women > 80 years of age	0.98	NR	NR
Vega-Hinojosa, 2018 [21] cross-sectional	COPCORD, Peru	1095	481	614	Men and women ≥ 18 years of age	1.55	NR	NR

*$p = 0.001$ vs. men
**$p < 0.01$ vs. men
***$p = 0.02$ vs. men
COPCORD, Community Oriented Program for Control of Rheumatic Diseases; *NR*, not reported; *OA*, osteoarthritis

In Mexico the OA prevalence is about 19.6% (Clinical Reumathology, 2022)[6], the highest prevalence in the Latin American countries studied.

Osteoarthrosis symptoms can usually be managed, although the damage to joints is not easy to be reversed. Staying active, maintaining a healthy weight and receiving certain treatments might slow progression of the disease and help improve pain and joint function.

2.3 Risk factors and pathophysiology

Factors that can increase the risk of osteoarthrosis include[7]:

Older age: The risk of osteoarthritis increases with age.

Sex: Women are more likely to develop osteoarthritis, though it isn't clear why.

Obesity: Carrying extra body weight contributes to osteoarthritis in several ways, and the more you weigh, the greater the risk. Increased weight adds stress to weight-bearing

joints, such as your hips and knees. Also, fat tissue produces proteins that can cause harmful inflammation in and around the joints.

Joint injuries: Injuries, such as those that occur when playing sports or from an accident, can increase the risk of osteoarthritis. Even injuries that occurred many years ago and seemingly healed can increase the risk of osteoarthritis.

Repeated stress on the joint. If some job or sport places repetitive stress on a joint, that joint might eventually develop osteoarthritis.

Genetics: Some people inherit a tendency to develop osteoarthritis.

Bone deformities: Some people are born with malformed joints or defective cartilage.

Certain metabolic diseases: These include diabetes and a condition in which your body has too much iron (hemochromatosis).

Osteoarthrosis is a degenerative disease that worsens over time, often resulting in chronic pain. Joint pain and stiffness can become severe enough to make daily tasks difficult. Depression and sleep disturbances can result from the pain and disability of Osteoarthrosis.

Osteoarthrosis occurs when the cartilage that cushions the ends of bones in the joints gradually deteriorates. Cartilage is a firm, slippery tissue that enables nearly frictionless joint motion. Eventually, if the cartilage wears down completely, bone will rub on bone.

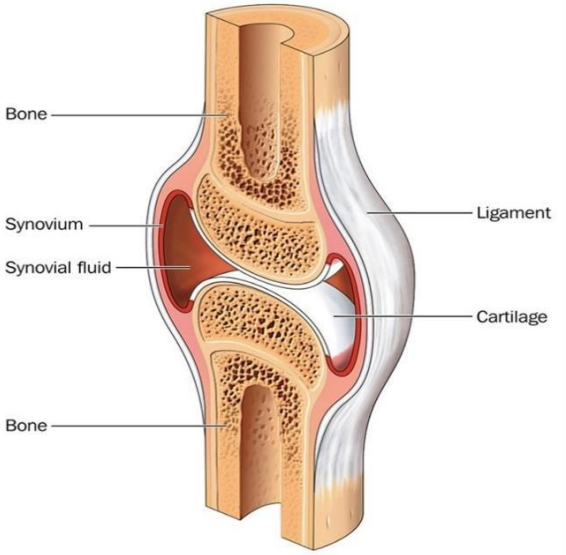
Osteoarthrosis has often been referred to as a wear and tear disease. But besides the breakdown of cartilage, Osteoarthrosis affects the entire joint. It causes changes in the bone and deterioration of the connective tissues that hold the joint together (tendons and ligaments) and attach muscle to bone. It also causes inflammation of the joint lining.

Cartilage structure and alterations

Cartilage is a strong, flexible connective tissue that protects joints and bones. It acts as a shock absorber throughout the body. Cartilage at the end of bones reduces friction and prevents them from rubbing together. It is also the main tissue in some parts of the body and gives them their structure and shape[8].

Cartilage is found in many areas: joints between bones, e.g. the elbows, knees and ankles, ends of the ribs, between the vertebrae in the spine, ears and nose, bronchial tubes or airways.

Cartilage is made up of specialized cells called chondrocytes. These chondrocytes **produce large amounts of extracellular matrix composed of collagen fibers, proteoglycan, and elastin fibers.** There are no blood vessels in cartilage to supply the chondrocytes with nutrients. Instead, nutrients diffuse through a dense connective tissue surrounding the cartilage (called the perichondrium) and into the core of the cartilage. Due to the lack of blood vessels, cartilage grows and repairs more slowly than other tissues.



Cross section through a typical synovial joint, showing the bone, synovial membrane, synovial fluid, cartilage and ligament, Image Credit: Blamb / Shutterstock

Cartilage types

- ***Hyaline cartilage -*** This is a low-friction, wear-resistant tissue present within joints that is designed to bear and distribute weight. It is a strong, rubbery, flexible tissue but has a poor regenerative capacity.

- ***Elastic cartilage -*** Elastic cartilage is more flexible that hyaline cartilage and is present in the ear, larynx and epiglottis.

- ***Fibrocartilage -*** Fibrocartilage is a tough and inflexible form of cartilage found in the knee and between vertebrae.

- ***Articular cartilage -*** Articular cartilage is the hyaline cartilage that lies on the surface of bones. This cartilage is often described in terms of four zones between the articular surface and the subchondral bone which include:

1. The surface or superficial tangential zone

This cartilage covers the articular surface and has a smooth contour that allows gliding of the ends of the bones and resists shear. It forms around 10% to 20% of articular cartilage thickness and has the highest collagen content of all the zones. The collagen fibrils are densely packed and are aligned in a highly organized manner parallel to the articular surface. The chondrocytes in this zone are elongated in shape.

2. The middle (or transitional) zone

The middle zone makes up 40% to 60% of the articular cartilage volume. The collagen fibrils are thicker and aligned loosely and not in parallel to the surface. Chondrocytes in this layer are more rounded.

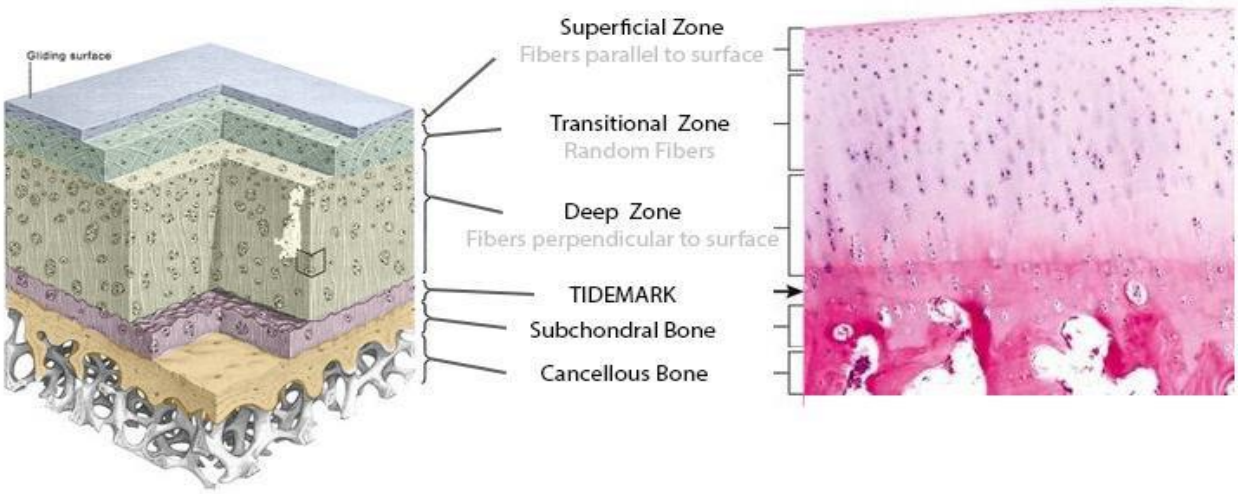
3. The deep zone

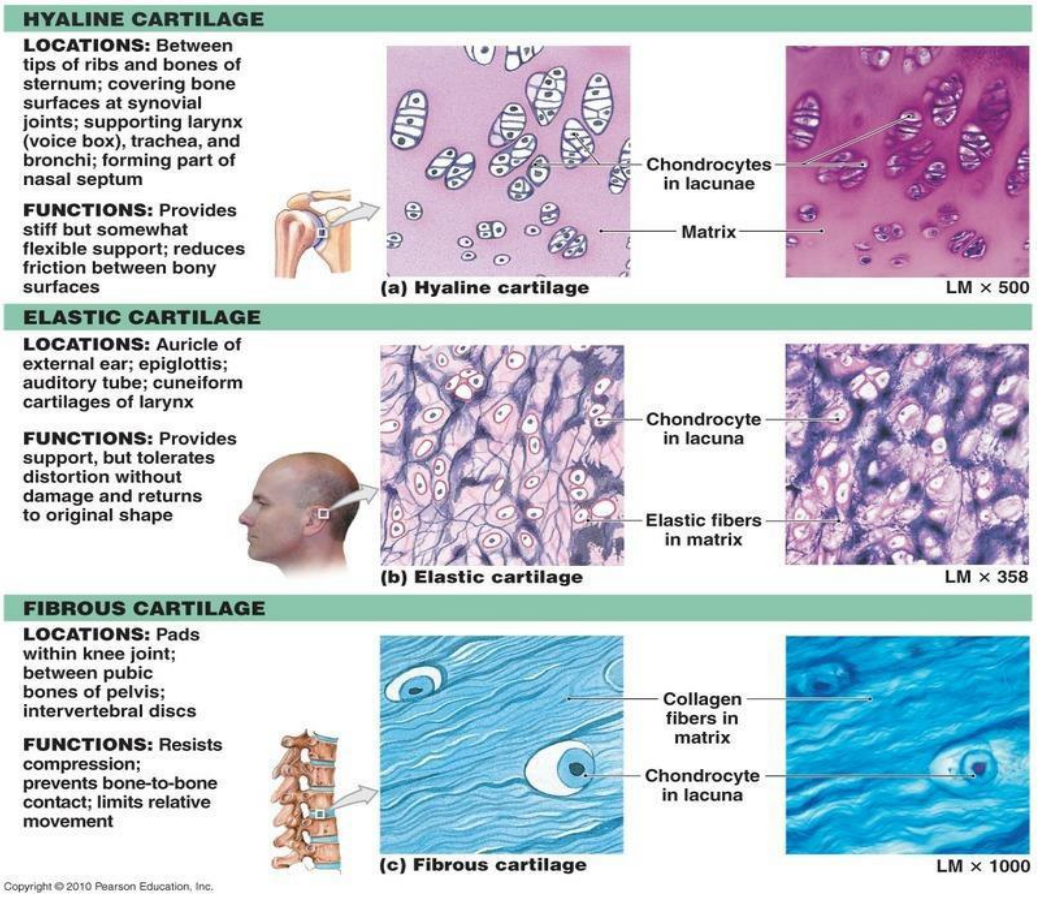
This makes up 30% of the cartilage. The collagen fibrils are large in diameter and aligned perpendicular to the articular surface. This layer has the highest proportion of proteoglycan and lowest concentration of water. The chondrocytes are arranged in a columnar fashion, parallel to the collagen fibers.

4. The calcified zone

This lies directly on the subchondral bone and contains small cells in a chondroid matrix that has apatitic salts scattered through it.

Source: https://www.orthobullets.com/basic-science/9017/articular-cartilage

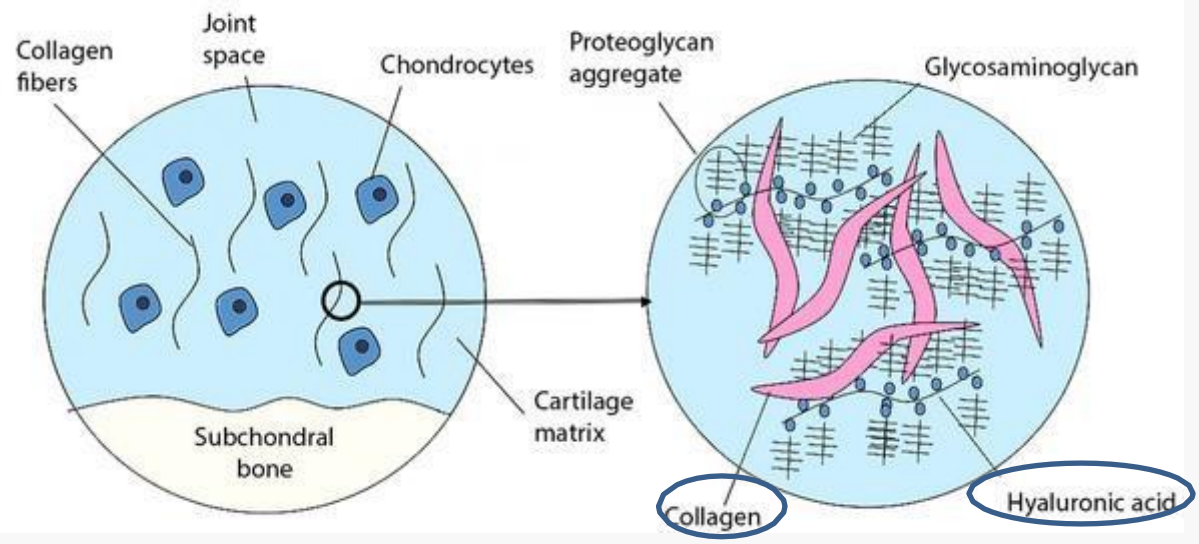




cartilage types - Bing images

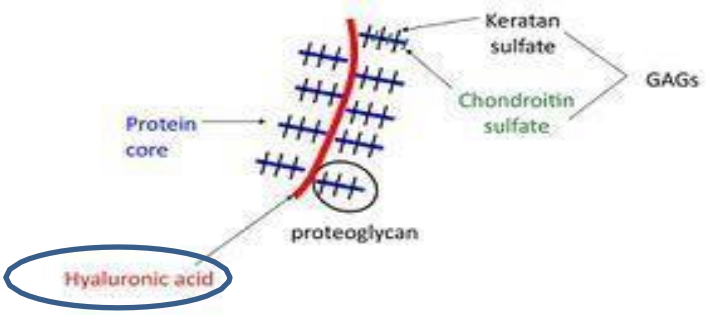
Proteoglycans

These molecules are of a class of glycoproteins of high molecular weight that are found especially in the extracellular matrix of connective tissue (the fibrous tissue that gives support to the body structure). Proteoglycans make up a major part of the extracellular matrix, filling the spaces that occur between cells. Different from other body tissues, the extracellular matrix (ECM) is the most significant part of connective tissue[9].



GAGs, proteoglycan, glycoproteins, cartilage matrix

https://www.physio-pedia.com/Proteoglycans



GAGs, proteoglycan, glycoprotein, keratan sulfate, cartilage matrix

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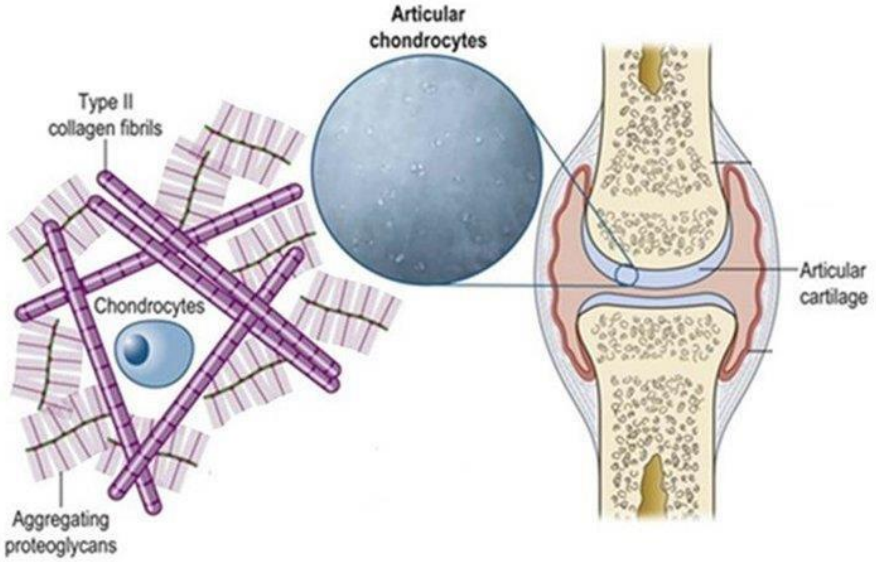


https://www.physio-pedia.com/Proteoglycans

Proteoglycans classification

Nature of GAGs chains: Proteoglycans can be classified according to the glycosaminoglycans (GAGs) attached to them. The types are chondroitin sulfate, dermatan sulfate, heparin sulfate, heparan sulfate, or keratan sulfate.

Proteoglycans are categorized by their relative size (large and small): Large molecules e.g. aggrecan, an important part of cartilage, versican, which is found in the blood vessels and skin. Small molecules eg decorin, biglycan, fibromodulin, and lumican. **Proteoglycans are a major component of the ECM, being the filler** between cells in an organism. Proteoglycans have an important role in the physiology and biomechanical function of tendons, ligaments and cardiovascular system via their involvement in regulation of assembly and maintenance of ECM, and as they participate in cell proliferation through their interactions with growth factors; eg. **Aggrecan provides cartilage with the property to bind with water to form hydrated matrices. These molecules act as fillers between the cell spaces.**

Proteoglycans in pathophysiology of osteoarthrosis

The loss of proteoglycans from articular cartilage is a hallmark of the osteoarthritic process. Compensatory biosynthesis of proteoglycans is evident by histochemical analysis, characterized by significant metachromasy in the pericellular region of the matrix, at a time when proteoglycans are apparently lost from the interterritorial matrix. Changes, occurring in the pericellular region demonstrating a loss

in metachromasy, suggest that **depletion of specific proteoglycans surrounding the chondrocyte may be of significance in the early osteoarthritic lesion**. Organ culture studies of human and animal cartilage indicated that synthesis of proteoglycans occurs normally, but **that post-synthesis processing, allowing for efficient formation of proteoglycan aggregates, may be impaired**. In addition, **recent experiment data are consistent with proteolytic degradation of newly synthesized proteoglycan core protein which significantly reduces proteoglycan aggregate formation.**

2.4 Clinical symptoms, diagnosis and complications

The **main symptom is pain**, causing loss of ability and often stiffness. The pain is typically made worse by prolonged activity and relieved by rest. **Stiffness** is most common in the morning, and typically lasts less than thirty minutes after beginning daily activities, but may return after periods of inactivity. Osteoarthrosis can cause a crackling noise (called "crepitus") when the affected joint is moved, especially shoulder and knee joint. A person may also complain of **joint locking and joint instability**. These symptoms would affect their daily activities due to pain and stiffness. Some people report increased pain associated with cold temperature, high humidity, or a drop in barometric pressure, but studies have had mixed results.

As osteoarthrosis progresses, movement patterns (such as gait), are typically affected. Osteoarthrosis is the most common cause of a joint effusion of the knee.

In smaller joints, such as at the fingers, hard bony enlargements, called Heberden's nodes (on the distal interphalangeal joints) or Bouchard's nodes (on the proximal

interphalangeal joints), may form, and though they are not necessarily painful, they do limit the movement of the fingers significantly. Osteoarthrosis of the toes may be a factor causing formation of bunions, rendering them red or swollen.

Symptoms overview

Pain. Affected joints might hurt during or after movement.

Stiffness. Joint stiffness might be most noticeable upon awakening or after being inactive.

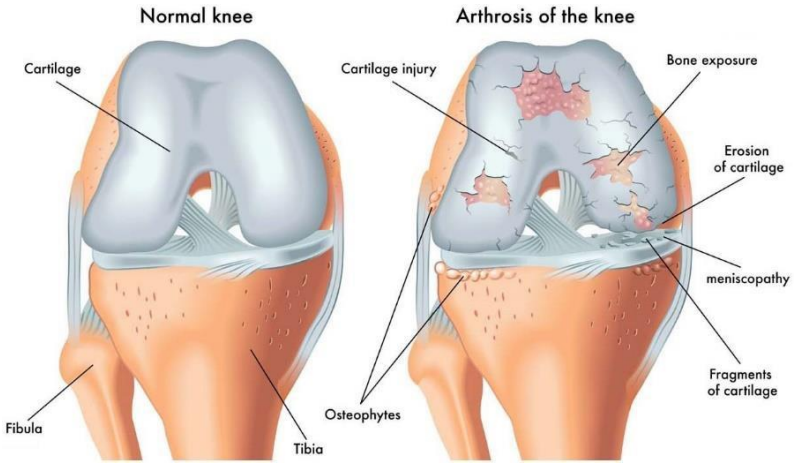
Tenderness. When it is applied light pressure to or near the joint.

Loss of flexibility. The joint can't move through its full range of motion.

Grating sensation. a grating sensation when using the joint, and it is possible to hear popping or crackling.

Bone spurs. Extra bits of bone can form around the affected joint leading pain

Swelling. This might be caused by soft tissue inflammation around the joint.



https://www.local-physio.co.uk/articles/general/osteoarthritis

Osteoarthrosis diagnosis

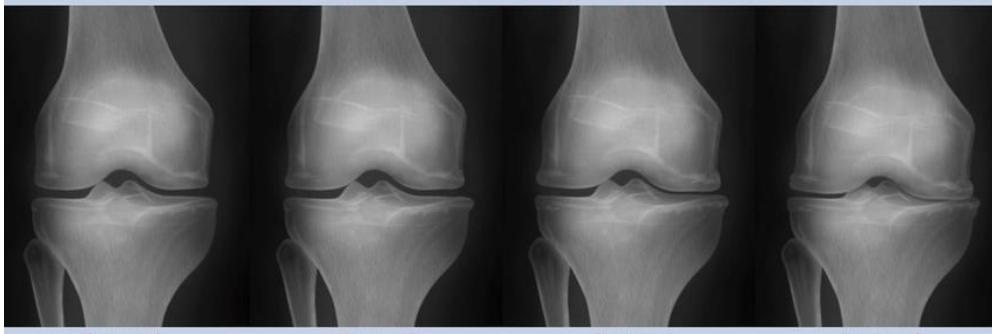
OA can be diagnosed radiographically or clinically. Although pathological changes may be evident in all structures within an OA joint, articular cartilage abnormalities are always present. Because of the ease of standardization and acquisition, radiography is the gold standard for diagnosing OA using the Kellgren-Lawrence (KL) grading system. This system has been mostly used for hand, hip, and tibiofemoral joint OA as a semiquantitative assessment, measuring OA severity on a scale of 0 to 4, with >2 defining radiographic OA. However, the KL grading system has limitations when assessing early-stage OA with only mild cartilage abnormalities or a localized cartilage defect. Magnetic resonance imaging (MRI) is more sensitive during these earlier stages as it can image soft tissue structures including articular cartilage, meniscus, ligaments, bone marrow, labrum, and synovium. It can also detect the changes in articular cartilage composition that occur before morphologic changes[10].

However, MRI is currently still not a standard technique to diagnose and monitor OA. Numerous variations of the **Kellgren and Lawrence classification** system have been used in research. Below is the original description:

- **grade 0 (none):** definite absence of x-ray changes of Osteoarthrosis
- **grade 1 (doubtful):** doubtful joint space narrowing and possible osteophytic lipping
- **grade 2 (minimal):** definite osteophytes and possible joint space narrowing
- **grade 3 (moderate):** moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
- **grade 4 (severe):** large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends

Osteoarthrosis is deemed present at grade 2 although of minimal severity.

Kellgren-Lawrence (KL) grading scale



Grade 1 Grade 2 Grade 3 Grade 4

CLASSIFICATION	Normal	Doubtful	Mild	Moderate	Severe
DESCRIPTION	No features of OA	Minute osteophyte: doubtful significance	Definite osteophyte: normal joint space	Moderate joint space reduction	Joint space greatly reduced: subchondral sclerosis

Source: https://www.researchgate.net/figure/The-Kellgren-and-Lawrence-grading-system-to-assess-the-severity-of-knee-OA-Source_fig3_339941257

Osteoarthrosis complications include:

- Rapid, complete breakdown of cartilage resulting in loose tissue material in the joint (chondrolysis).
- Bone death (osteonecrosis).
- Stress fractures (hairline crack in the bone that develops gradually in response to repeated injury or stress).
- Bleeding inside the joint.
- Infection in the joint.
- Deterioration or rupture of the tendons and ligaments around the joint, leading to loss of stability.
- Pinched nerve (in osteoarthritis of the spine).

2.5 Arthrosis treatment and guidelines

The goals of treatment for OA of the knee include relief of pain and inflammation, reduction of stiffness and improvement or preservation of range of motion, and improvement in or maintenance of mobility, function, and health-related quality of life[11].

Treatment options for OA include those in the following list:

- **Analgesics (oral, intra-articular, or topical) and anti-inflammatory agents** (nonsteroidal anti-inflammatory drugs [NSAIDs], **intraarticular corticosteroids**);

OARSI guidelines on NSAIDs

- Topical non-steroidal anti-inflammatory drugs (NSAIDs) are strongly recommended for individuals with Knee OA (Level 1A).
- Patients on anticoagulants or

OARSI guidelines on Corticosteroids

- provide moderate evidence for short-term pain reduction and improvement in function
- have multiple potential adverse effects (reduction in immune function, increased risk of

- **Dietary supplements** (including glucosamine with or without chondroitin and herbal mixtures), variously proposed to control pain and possibly serve as disease-modifying agents;

- **Physical treatments** (including strength or aerobic exercise, physical therapy, stretching, heat, aqua-therapy, whole-body vibration, electrical stimulation therapies (neuromuscular electrical stimulation [NMES] and transcutaneous electrical nerve stimulation [TENS]), massage, and chiropractic manipulation), proposed to strengthen muscles that support the affected joints and to increase range of motion;

- **Weight loss** to decrease the stress on the joint;

- **Intra-articular viscosupplementation,** which involves local injections of the natural joint lubricant, **hyaluronic acid (IA-HA).**

Sodium hyaluronate is a pharmaceutical treatment that has been commonly used for moderate OA in recent years. Because the concentration and chain length of synovial hyaluronan have been observed to be low in OA patients, **intraarticular injections of sodium hyaluronate improve shock absorption by replenishing sodium hyaluronate in the joint cavity.** Unfortunately, the effects of these injections need to be supported by controlled clinical trials on each type of HA. In clinical practice, the decision to use hyaluronic acid injections may be regarded more favorably than offering no intervention for OA patients whose response to anti-inflammatory treatment has been insufficient. An intraarticular injection agent that can be used as an alternative to sodium

hyaluronate is **crosslinked sodium hyaluronate, which has a higher molecular weight and greater stability and viscosity than unmodified sodium hyaluronate** due to crosslinking. Therefore, this crosslinked agent may be expected to have a longer duration of action than hyaluronate. Sorbitol and mannitol which have intrinsic free radical scavenger properties have been the most studied antioxidants. **Sodium hyaluronate and polyols** develop together a complex based on a dense network of hydrogen bonds which do not modify the viscoelastic properties of hyaluronic acid. **The oxygen free radicals neutralization by mannitol has been proven to delay the degradation of both linear and cross-linked HA in several in vitro models of oxidative stress**. The antioxidant effect of these polyols may also play a role in accelerating onset of analgesia, as demonstrated in a double-blind controlled trial comparing a mannitol-modified medical device to regular hyaluronic acid. The addition of mannitol to hyaluronic acid does not alter the safety and local tolerability. In summary, leading to a faster effect on pain relief without increasing the risk of adverse effect.

IA-HA important tips from Guidelines and EBM[11]:

- **IAHA efficacy varies widely across preparations.** High-quality studies are required to assess and compare the safety and efficacy of IA-HA preparations.
- **adding a polyol, such as mannitol, to hyaluronic acid may improve the effects of viscosupplementation** by reducing the rate of degradation of HA
- **IAHA is recommended with a Level 1B/Level 2 treatment for Knee OA,** dependent upon comorbidity status

- **Injections of platelet-rich plasma (PRP),** are aimed at reversing or slowing the progression of the disease. A recent metanalysis (Han et al. 2019) comparing PRP vs HA injection in patient with knee OA showed that long t

pain relief and functional improvement in the PRP group were superior to those in the HA group. The data from 15 RCTs showed that long term pain relief and functional improvement in the PRP group were superior to those in the HA group. Subjects in the PRP group experienced significantly better pain relief (WOMAC and VAS pain score) and functional improvement (WOMAC functional and total score, IKCD score) between 6-12 months post-injection than those in the HA group.

PRP has a better effect on patients with early to moderate forms of KOA (knee osteoarthrosis) but has a limited effect on patients with advanced form of KOA.

PRP limitations[12]

- We cannot recommend the routinary use of PRP injection as a treatment for OA because **composition remain uncertain**: the preparations differ in the platelet counts, GFs concentration, leukocyte counts depending on the patient characteristics and preparation kit
- **The interval between injections and optimal dosage are still areas of concern** in the future because they might exert an effect on the post-injection process, and there are currently no published studies supporting any specific injection protocol.

- **Surgical procedures,** including arthroscopy with lavage and/or debridement, and partial or total arthroplasty (eg. knee replacement), which may be recommended for advanced cases if patients fail to obtain satisfactory relief from pain and improved function from the aforementioned treatments.

CHAPTER 3

CONDROTIDE HA INTRODUCTION

3.1 Condrotide HA description

Condrotide HA is an injectable medical device, formulated as sterile, non-pyrogenic, viscoelastic solution supplied in a pre-filled syringe for single use.

a. Characterizing compounds

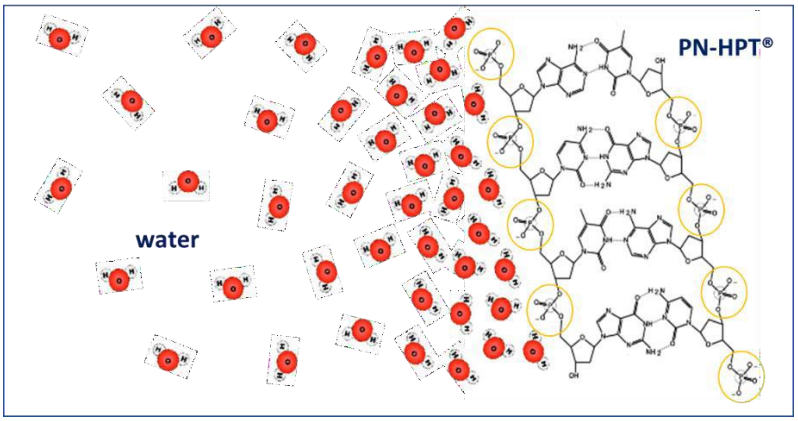
The characterizing components of Condrotide HA are **Polynucleotides (PN)** at a concentration of 10 mg/ml **and Hyaluronic acid sodium salt** at a concentration of 10 mg/ml. It also contains **mannitol.**

b. Main characteristics and biological action

Polynucleotides are **polymeric molecules** which are able to bind a large amount of water and to re-organize their structure by orienting and coordinating water molecules to form a 3-D gel.

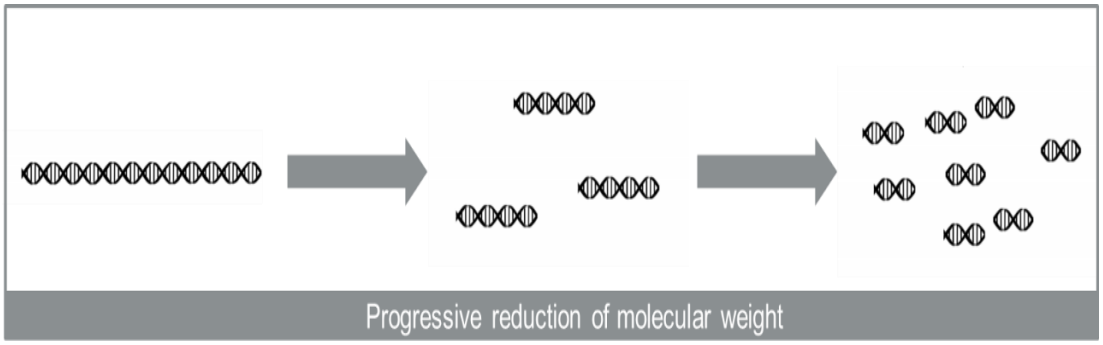
In the short-term, after intra-articular infiltration, these natural-origin polymers can **deeply moisturize articular surfaces**.

PN short-term action



In the long term, polynucleotides undergo enzymatic cleavage and **progressively release in the articular cavity both water molecules and smaller-sized oligonucleotides** that retain their moisturizing and viscoelastic properties **thereby maintaining the effect longer.**

PN long term action



Polynucleotides are extracted from natural sources (fish-trout sperm). It is already known in literature that the derivatives of enzyme degradation of polynucleotide chains (simple nucleotides, nucleosides, nitrogen bases) are physiologically present in the extra-cellular environment and are useful substrates for cells. **Intra-articular infiltration progressively enriches the synovial fluid of PN and thus of nucleotides, purine and pyrimidine bases that cells (eg. chondrocytes and mesenchymal cells) use to promote their anabolic activity**[13].

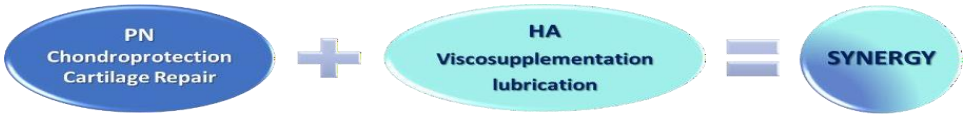
Mastelli Srl, introduced in the market the first medical devices with polynucleotides based on top-standard biotechnologies, refined over 60 years.

"High Purification Technology" is applied by Mastelli to extract DNA fragments and to obtain a "product by process": highly concentrated and purified without pharmacologically and allergically active protein contaminants, by means of high temperature sterilizing procedures.

Notably, Mastelli is the first company to control the entire production chain, in compliance with worldclass GMP, GDP and QA standards, ensuring the safety of the finished product, particularly important aspect for animal derived products.

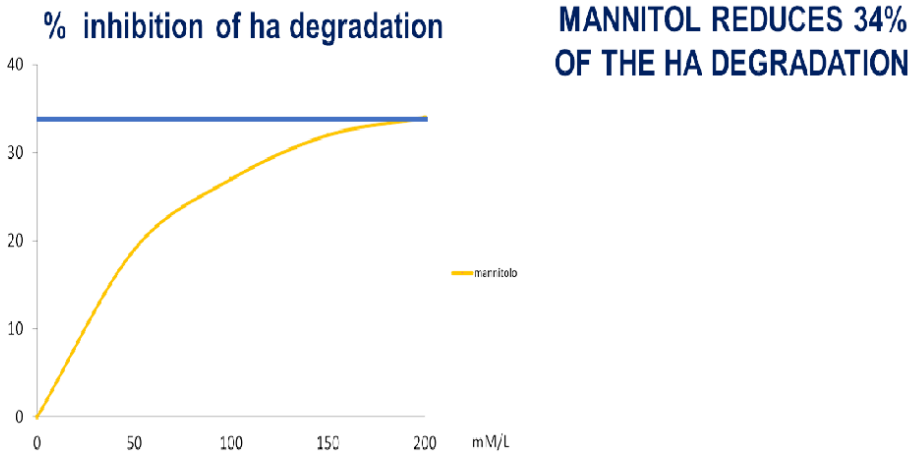
Nowadays Mastelli distributes PN-HPT™- based products **in over 30 countries all over the world, with a rigorous safety surveillance system.**

Condrotide HA also contains **Hyaluronic acid of biofermentative origin to induce a synergistic effect.**



Mannitol is a polyol, which develops an antioxidant activity by scavenging Radicalic Oxygen Species "ROS". Several studies have evidenced the role of mannitol in the protection of HA against ROS and hyaluronidase enzymes. In Plinart HA **mannitol**[14]:

- **protects hyaluronic** acid **from enzymatic degradation and oxidative stress**
- **slows the breakdown of hyaluronic** acid and thus **prolongs the action** in time



c. Licensed indications-Therapeutic use

The product is **indicated for painful joint diseases** caused by degenerative or post-traumatic conditions or by modification of a joint. **Condrotide HA is a temporary synovial fluid substitute that thanks to its viscoelastic and lubricating properties** helps to restore the rheological conditions of the joints. **Since the product improves the characteristics of the synovial fluid, it has a protective action on the joints and promotes the physiological mechanisms of repair of the articular cartilage.**

3.2 Condrotide HA: posology and way of administration

The recommended treatment consists of 3 injections in the joint to be treated. The time interval between each injection is 1-3 weeks.

Condrotide HA must be administered by injecting the solution in the intra-articular space with a sterile needle whose gauge varies between 18G and 22 G, usually being 20G. Given the viscosity of the solution, the needle must be firmly screwed onto the Luer-Lock fitting of the syringe to ensure that the connection is watertight, preventing any leakage between the needle and the syringe when administering the product. This intra-articular injection must be performed by medical personnel only, complying with the aseptic procedures required for this method of administration. The product must be injected inside the joint cavity. The injection point must be on the healthy skin.

3.3 Condrotide HA: contraindications and special warnings

The use of the **product is contraindicated in patients who are hypersensitive to any of the product's components or with a positive anamnesis for allergy to products of fish origin.**

Since the effectiveness and the safety of Condrotide HA have not been proved in pregnant or breastfeeding women or in children, treatment with Condrotide HA is advised against in these cases.

Avoid injection if the joint is infected or seriously inflamed. To prevent the possible onset of bacterial arthritis avoid injection in patients with on-going infections or inflammatory condition of the skin close to the site of injection. The product must be used with particular care in patients with lymphatic or venous stasis of the leg to be treated. In case of joint effusions, it is advisable to remove the fluid by aspiration before injecting the product. Do not inject via the intravenous route. Patients receiving intra-articular injections should be advised to avoid any demanding forms of physical exercise for the joint and to resume their normal activities after a couple of days. Since Condrotide HA is a product for single use, any residue must be eliminated.

CHAPTER 4

CONDROTIDE EFFICACY AND CLINICAL BENEFITS ON DAMAGED JOINTS

4.1 Main effects on damaged articulations

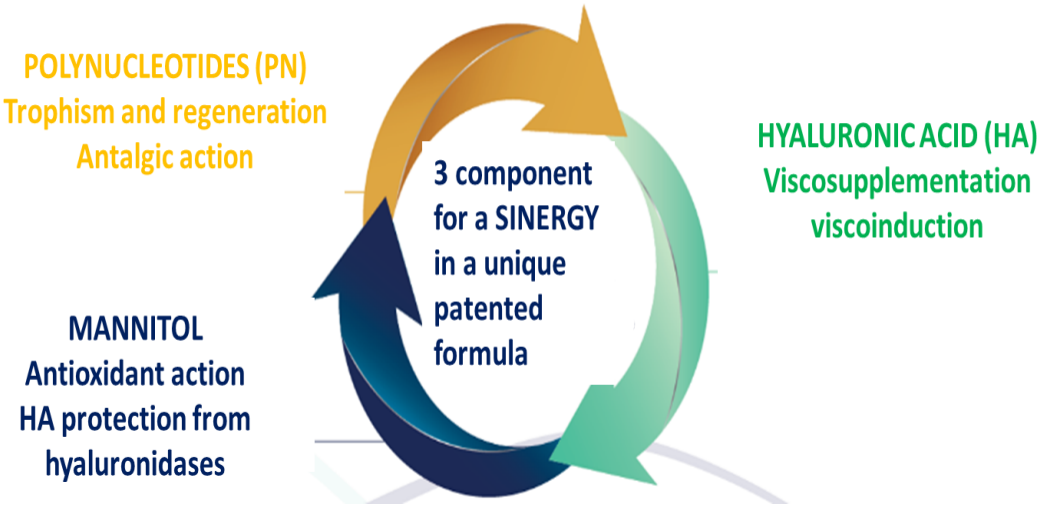
The main purpose of Polynucleotides provided with Condrotide HA treatment is to promote at intra-articular level the chondrocytes survival and a significant increase of production of cartilage extracellular matrix protein (ECM), including Aggrecan and Collagen II. In synergy with polynucleotides, hyaluronic acid will improve the hydration and viscosupplementation and mannitol exerts an antioxidant-antiradicalic protective action and also prolonges the hyaluronic acid activity by inhibiting hyaluronidase enzymes[15].

All these **effects at the joint level** are aimed to:

- **relieve pain and improve** articular **function**;
- **slow** the disease **progression**;
- **reduce** the intake of **analgesics and anti-inflammatory** drugs
- **improve** patient's **quality of life**

Condrotide HA: 3 combined actions

1. Vanelli R, Costa P, Rossi SM, Benazzo F. Efficacy of intra-articular polynucleotides in the treatment of knee osteoarthritis: a randomized, double-blind clinical trial. Knee Surg Sports Traumatol Arthrosc. 2010;18(7):901-7
2. Giarratana LS, Marelli BM, Crapanzano C, De Martinis SE, Gala L, Ferraro M, Marelli N, Albisetti W. A randomized double-blind clinical trial on the treatment of knee osteoarthritis: the efficacy of polynucleotides compared to standard hyaluronian viscosupplementation. The Knee. 2014;21(3):661-8
3. Saggini R, Di Stefano A, Capogrosso F, Carniel R, Haidar Hassan K, Bellomo RG. Viscusupplementatiomn with Hyaluronic Acid or Polynucleotides: Results and Hypothesis for Condro-synchronization. J Clin Trials 2014; 4(6)
4. F Gennero L, Denysenko T, Calisti GF, Vercelli A, Vercelli CM, Amedeo S, Mioletti S, Parino E, Montanaro M, Melcarne A, Juenemann C, De Vivo E, Longo A, Cavallo G, De Siena R. Protective effects of polydeoxyribonucleotides on catilage degradation in experimental cultures Cell Biochemistry and Function 2013;31(3):214-27



4.2 In vitro studies

The in vitro models are useful to research, find and apply an appropriate microenvironment to cartilage recovery. The first in-vitro study (Gennero L., 2013) was focused on the research of a proper microenvironment to re-induce the cell physiological functions in injured cartilage. Different culture media (Polynucleotides, HA, laboratory culture medium specific for cartilage and negative controls) were tested on explants of cartilage or derived cells. HA represents a positive control with clearly established in vivo efficacy. To date, it is the main device used in vivo to restore the appropriate intra-articular viscosity. Laboratory culture medium specific for cartilage represents an elective positive control for in vitro activity. It is generally used for in vitro cartilage cell culture. Negative controls were prepared consisting of non-specific cell culture media or cell culture media without supplements. PNs, positive controls and negative controls were compared to promote the eutrophy of chondrocytes and a correct ECM deposition.

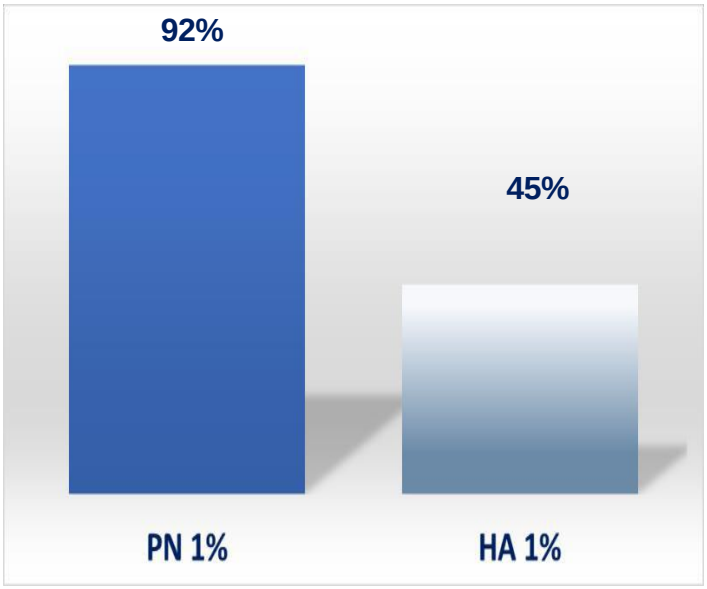
Cartilage Biopsies and cells were maintained in different culture media for 30 days. The viability of extracted cells was evaluated by trypan blue and MTT staining. At day 0 of

incubation, the viability and the morphology of cells were found equal both in all samples and in all controls. At the 30th day of incubation, the viability of chondrocytes was significantly high in the 1% PN-treated samples versus positive control (1% HA)[16].

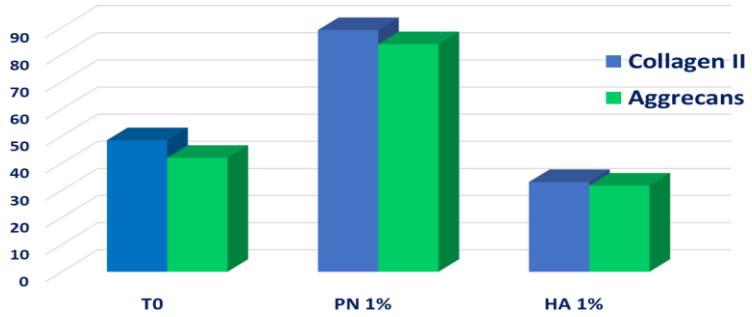
RESULTS:

- Significantly **higher chondrocytes survival** in all biopsies treated with PN-HPT™ vs those treated with HA (*P<0.001, after 30 days treatment);
- Significant **increase of extracellular matrix proteins** (collagen and aggrecans)

CELL SURVIVAL (%)



ECM DEPOSITION (%)



Final Comments: The chondrocytes extracted form cartilage biopsies showed normally functioning ECM deposition when cultured with 1% PN solution. **The immunohistochemical analysis of samples treated with 1% PN demonstrated that the deposition of ECM was similar to hyaline cartilage** (physiological constituent of articular cartilage), whereas in **the samples treated with 1% HA, the deposition of ECM was similar to fibrous cartilage, which has limited repair capabilities**.

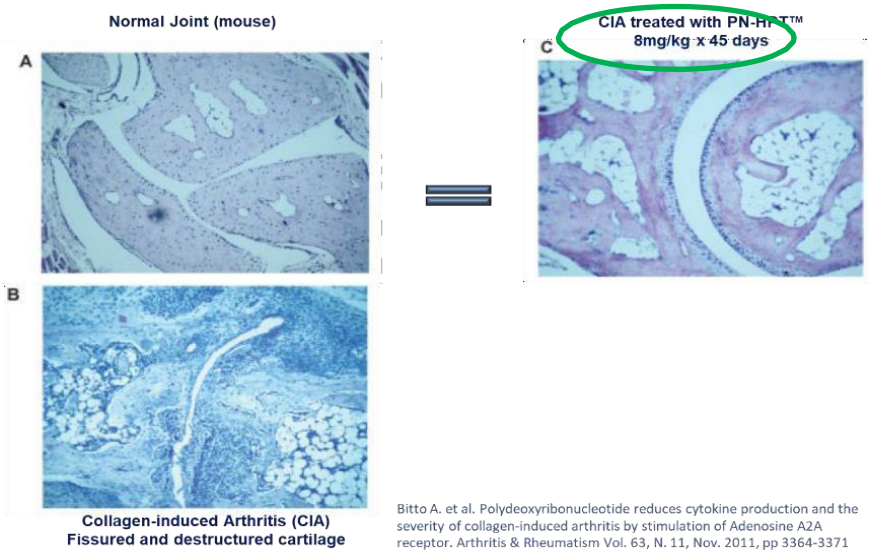
In another in vitro study were investigated the effects of Polynucleotides in collagen-induced arthritis (Bitto A. 2011). Arthritis was induced in DBA/1 mice by an intradermal injection of 100 ml of bovine type II collagen in Freund's complete adjuvant. Mice were immunized a second time 21 days later. Control animals received 100 ml of a saline solution. **Animals with "Collagen Induced Arthritis" CIA** were **randomized to receive one of the following: vehicle (1 ml/kg) and Polynucleotide (8 mg/kg intraperitoneally daily)**; The treatment was initiated immediately after the second immunization and continued to day 45. Clinical evaluation of arthritis was performed

throughout the study. On day 45, the animals were killed and the severity of arthritis was evaluated histologically. At the end of the experimental period (on day 45), knee joints were removed from the hind limbs of the animals and subjected to immersion fixation for 48 hours in 10% neutral buffered formalin. Joints were decalcified in 10% EDTA for 2–3 weeks and embedded in paraffin. Paraffin-embedded sections of joints were cut and stained with hematoxylin and eosin for histologic assessment. Arthritis severity in histologic samples was determined by cumulative assessment of synovial inflammation. Samples were scored for synovial inflammation on a scale of 0–5, where 0 normal, 1 minimal, 2 mild, 3 moderate, 4 marked, and 5 severe. Severity of bone erosion was also scored on a scale of 0–5, where 0 = normal; 1 = minimal, not readily apparent on low magnification; 2 = mild, not readily apparent on low magnification; 3 = medium, apparent on low magnification; 4 = marked, apparent on low magnification; and 5 = marked, erosion/loss with evidence of bone disruption. This scoring scale was based on a previously published method. Seven days after the first immunization, animals began to show evidence of clinical inflammation in 1 or more hind paws. The first manifestation of disease was erythema of 1 or more ankle joints, followed by metatarsal and interphalangeal joints. The figure shows the incidence of CIA at the end of the experiment. In the group of mice with CIA that received vehicle, 95% of the animals developed arthritis by the end of the experiment. Administration of PN significantly attenuated the development of arthritis (35%)[17].

RESULTS:

Polynucleotides-HPT™ reduce:

- **histologic damage**
- **inflammatory manifestations** at cartilagineous level



Bitto A. et al. Polydeoxyribonucleotide reduces cytokine production and the severity of collagen-induced arthritis by stimulation of Adenosine A2A receptor. Arthritis & Rheumatism Vol. 63, N. 11, Nov. 2011, pp 3364-3371

4.3 Clinical trials

Several clinical studies (here below cited) are **published on efficacy and safety of Polynucleotides alone versus Hyaluronic acid in different joints** such as **knee**[13]**, shoulder**[18]**, hip**[19] **and ankle**[20]**.**

1. Vanelli R, Costa P, Rossi P, Benazzo F.

Efficacy of intra-articular ***Polynucleotides in the treatment of knee osteoarthritis****: a randomized, double-blind clinical trial. Knee Surg Sports Traumatol Arthrosc. 2010; 18:901–907*

1. *Giarratana LS, Marelli BM, Crapanzano C, De Martinis SE, Gala L, Ferraro M, Marelli N, Albisetti W. A randomized double-blind clinical trial on the* ***treatment of knee osteoarthritis:*** *The efficacy of polynucleotides compared to standard hyaluronian Viscosupplementation. 2014; 21:661–668*

*3. Saggini R, Di Stefano A, Capogrosso F, Carniel R, Haidar Hassan K, Bellomo RG. Viscosupplementation with Hyaluronic Acid or Polynucleotides: Results and Hypothesis for Condrosynchronization. J Clin Trials 2014; 4(6) (****Shoulder****)*

4. Migliore A, Graziano E, Martín LSM, Sorbino A, Raichi M, Boni G. Three-year management of ***hip osteoarthritis*** *with intra-articular polynucleotides: a real-life cohort retrospective study. J Biol Regul Homeost Agents. 2021*

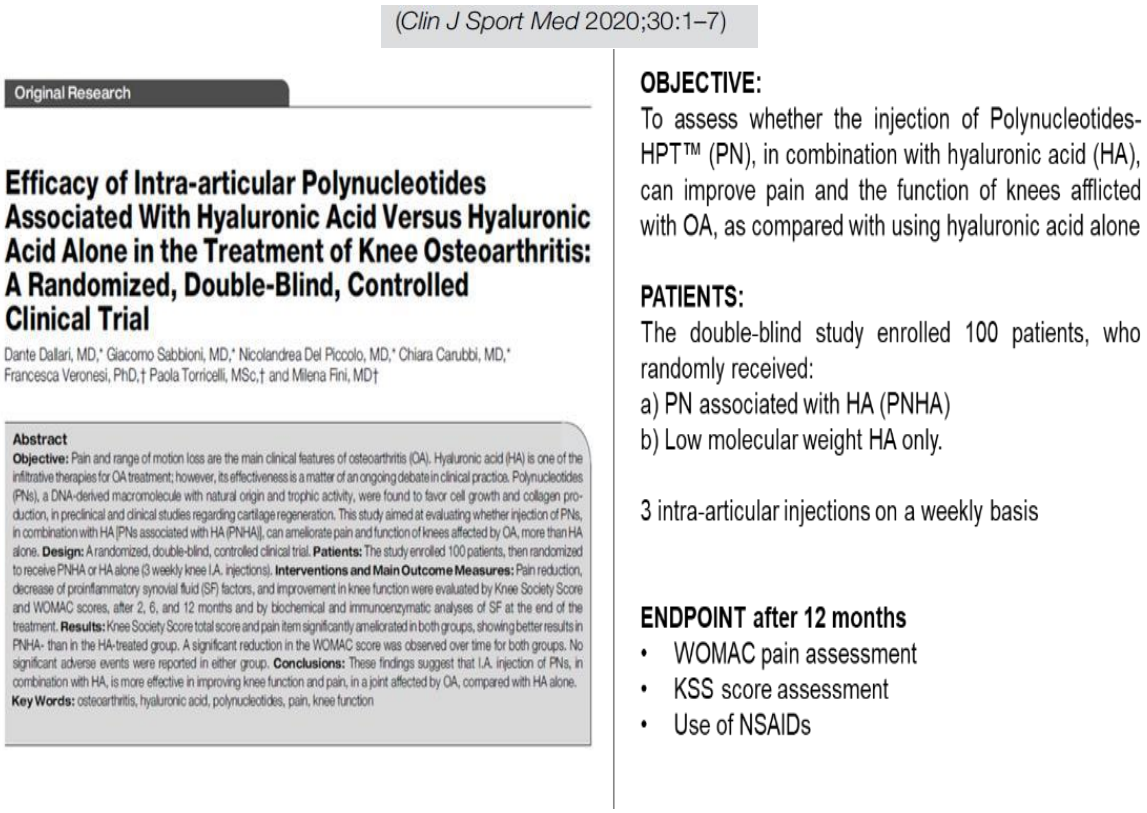
5. Guelfi M, Fabbrini R, Guelfi MG. Intra-articular treatment of ***knee and ankle*** *osteoarthritis with polynucleotides: prospective case record cohort vs. historical controls. Journal of Biological regulators & homeostatic agents. 2020*

All these studies demonstrated that **PNs lead to a reduction in OA symptoms, with an effect comparable or even better than HA, but also with an earlier and more prolonged response**.

On this premises we hypothesized that in patients affected by OA **the original association of PNs and HA injections (Condrotide HA) would reduce pain and improve function, more than HA alone up to 12-24 months. To confirm this hypothesis** we

summarize the results of **2 published clinical trials:**

1. ***Efficacy of Intra-articular Polynucleotides associated with hyaluronic acid versus hyaluronic acid alone in the treatment of knee osteoarthritis: a randomized, double-blind, controlled clinical trial (Dallari D. et al.)***



(*Clin J Sport Med* 2020;30:1–7)

Original Research

Efficacy of Intra-articular Polynucleotides Associated With Hyaluronic Acid Versus Hyaluronic Acid Alone in the Treatment of Knee Osteoarthritis: A Randomized, Double-Blind, Controlled Clinical Trial

Dante Dallari, MD,* Giacomo Sabbioni, MD,* Nicolandrea Del Piccolo, MD,* Chiara Carubbi, MD,* Francesca Veronesi, PhD,† Paola Torricelli, MSc,† and Milena Fini, MD†

Abstract

Objective: Pain and range of motion loss are the main clinical features of osteoarthritis (OA). Hyaluronic acid (HA) is one of the infiltrative therapies for OA treatment; however, its effectiveness is a matter of an ongoing debate in clinical practice. Polynucleotides (PNs), a DNA-derived macromolecule with natural origin and trophic activity, were found to favor cell growth and collagen production, in preclinical and clinical studies regarding cartilage regeneration. This study aimed at evaluating whether injection of PNs, in combination with HA [PNs associated with HA (PNHA)], can ameliorate pain and function of knees affected by OA, more than HA alone. **Design:** A randomized, double-blind, controlled clinical trial. **Patients:** The study enrolled 100 patients, then randomized to receive PNHA or HA alone (3 weekly knee I.A. injections). **Interventions and Main Outcome Measures:** Pain reduction, decrease of proinflammatory synovial fluid (SF) factors, and improvement in knee function were evaluated by Knee Society Score and WOMAC scores, after 2, 6, and 12 months and by biochemical and immunoenzymatic analyses of SF at the end of the treatment. **Results:** Knee Society Score total score and pain item significantly ameliorated in both groups, showing better results in PNHA- than in the HA-treated group. A significant reduction in the WOMAC score was observed over time for both groups. No significant adverse events were reported in either group. **Conclusions:** These findings suggest that I.A. injection of PNs, in combination with HA, is more effective in improving knee function and pain, in a joint affected by OA, compared with HA alone.

Key Words: osteoarthritis, hyaluronic acid, polynucleotides, pain, knee function

OBJECTIVE:
To assess whether the injection of Polynucleotides-HPT™ (PN), in combination with hyaluronic acid (HA), can improve pain and the function of knees afflicted with OA, as compared with using hyaluronic acid alone

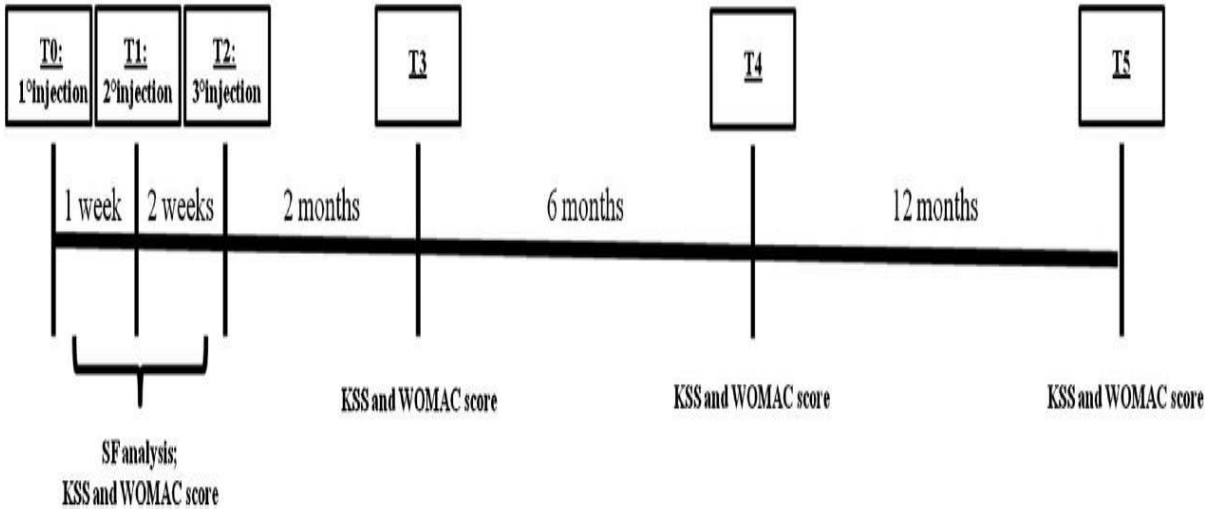
PATIENTS:
The double-blind study enrolled 100 patients, who randomly received:
a) PN associated with HA (PNHA)
b) Low molecular weight HA only.

3 intra-articular injections on a weekly basis

ENDPOINT after 12 months
- WOMAC pain assessment
- KSS score assessment
- Use of NSAIDs

Methods and experimental design:

This is a randomized, double-blind, controlled study, enrolling **98 patients affected** by **knee OA** (Kellgren-Lawrence mild to moderate, Table 1). **Were to be excluded from the study patients with pre-existing infiltrative therapies or patients with a single previous HA infiltration cycle, performed less than 6 months before enrollment.**



Endpoints of the study

- **WOMAC** improvement (**score have to decrease**): primary endpoint
- **KSS questionnaire** improvement (**score have to increase**): secondary endpoint

The *Western Ontario and McMaster Universities Arthritis Index (WOMAC)* is widely used in the evaluation of Hip and Knee Osteoarthritis. It is a self-administered questionnaire consisting of 24 items divided into 3 subscales:

- **Pain** (5 items): during walking, using stairs, in bed, sitting or lying, and standing upright
- **Stiffness** (2 items): after first waking and later in the day
- **Physical Function** (17 items): using stairs, rising from sitting, standing, bending, walking, getting in / out of a car, shopping, putting on / taking off socks, rising from bed, lying in bed, getting in / out of bath, sitting, getting on / off toilet, heavy domestic duties, light domestic duties.

  WOMAC Index was developed in 1982 at Western Ontario and McMaster Universities. WOMAC is available in over 65 languages and has been linguistically validated.

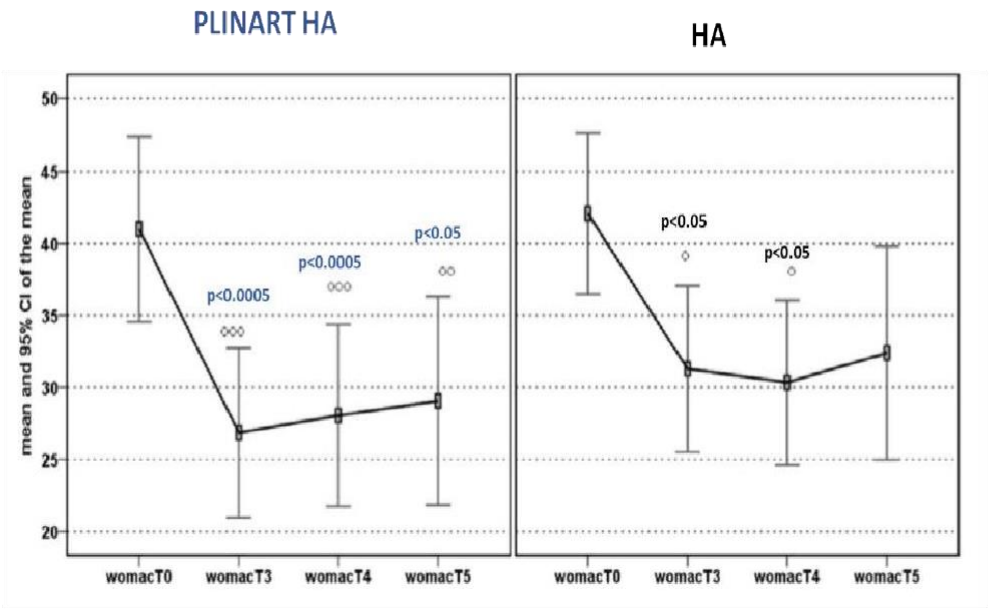
The Knee Society Score for Total Knee Replacement (KSS) contains questions in 2 sections: knee joint (pain, range of motion, stability) and function (walking distance, ability to climb stairs). When calculating the score, deductions are taken for assistive devices and flexion contractures, misalignment, or extension lag.

Results

- a **significant reduction in the WOMAC** score was observed **over time** in both groups.
- **KSS total score and pain** item significantly ameliorated in both groups, showing **better results in PN-HA** than in the HA-treated group
- **PN-HA improved** clinical aspects **at all experimental times**
- **HA reduced pain** after 2 and 4 months **but not after 12 months**[21]

Mastelli
At the origin of regeneration

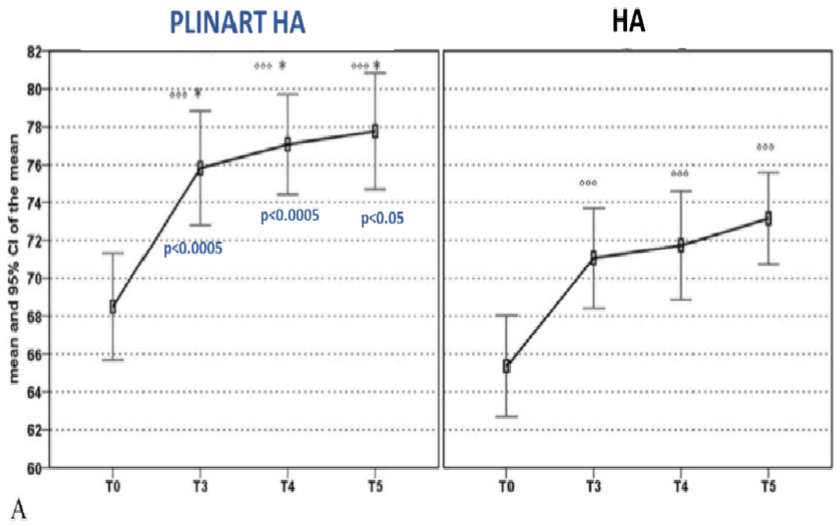
PAIN REDUCTION
WOMAC score



PLINART HA induced a significant **more reduction in pain over time *versus* HA alone**

PAIN, RANGE OF MOTION, JOINT STABILITY AND FUNCTION IMPROVEMENT

KSS score



A

PLINART HA induced a significant **more improvement in KSS SCORE over time *versus* HA alone** *(p<0,05 between treatments)*

1. ***Randomized, double-blind comparison of a fixed co-formulation of a fixed co-formulation of intra-articular polynucleotides and hyaluronic acid versus hyaluronic acid alone in the treatment of knee osteoarthritis: two-year follow-up (Stagni C. et al., 2021)***

This is a randomized, double-blind, controlled study, enrolling the same **98 patients affected** by **knee OA** (Kellgren-Lawrence mild to moderate, Table 1) as in the previous study **but with a follow-up prolonged up to 24 months.**

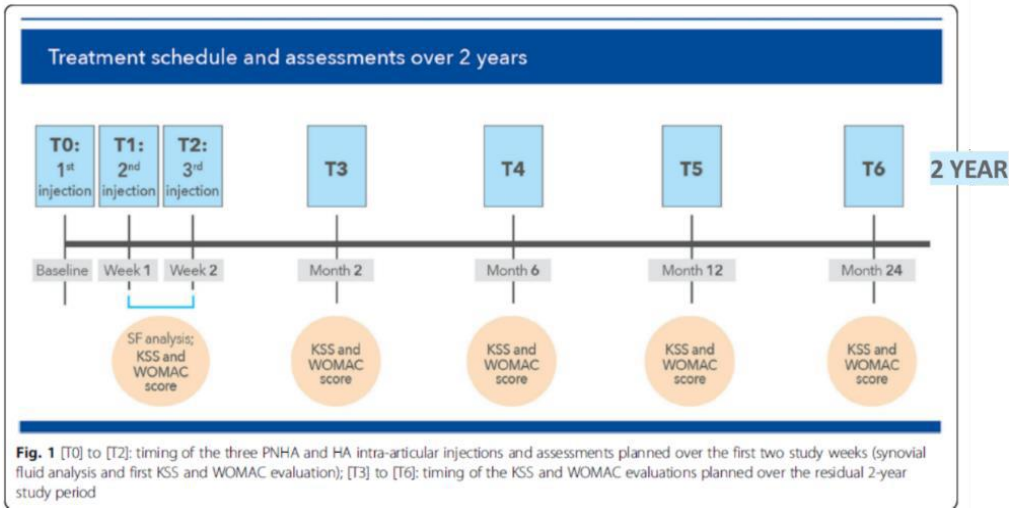


Fig. 1 [T0] to [T2]: timing of the three PNHA and HA intra-articular injections and assessments planned over the first two study weeks (synovial fluid analysis and first KSS and WOMAC evaluation); [T3] to [T6]: timing of the KSS and WOMAC evaluations planned over the residual 2-year study period

The **study aimed to verify over 2 years** whether:

- the **association of PN-HPT and HA injections would reduce pain and improve function** more than HA alone, as suggested by the authors in their first-year interim report;

- the **clinical synergy between PN-HPT™ and HA**, which the first-year interim analysis suggested, is persistent **over a much longer time** or just a transient medium-term effect.

Results

- **WOMAC pain score** already improved after 2 months in PN-HPT group. The **mean difference in favour of PN-HA** group *vs* HA control group was **about 16% over the 2-year follow-up period**
- **WOMAC item "walking on a flat surface"** improved significantly at T5 and T6
- **KSS score** were always higher in the PN-HA study group compared with the HA control group at all follow-up assessments.

WOMAC pain score difference vs baseline

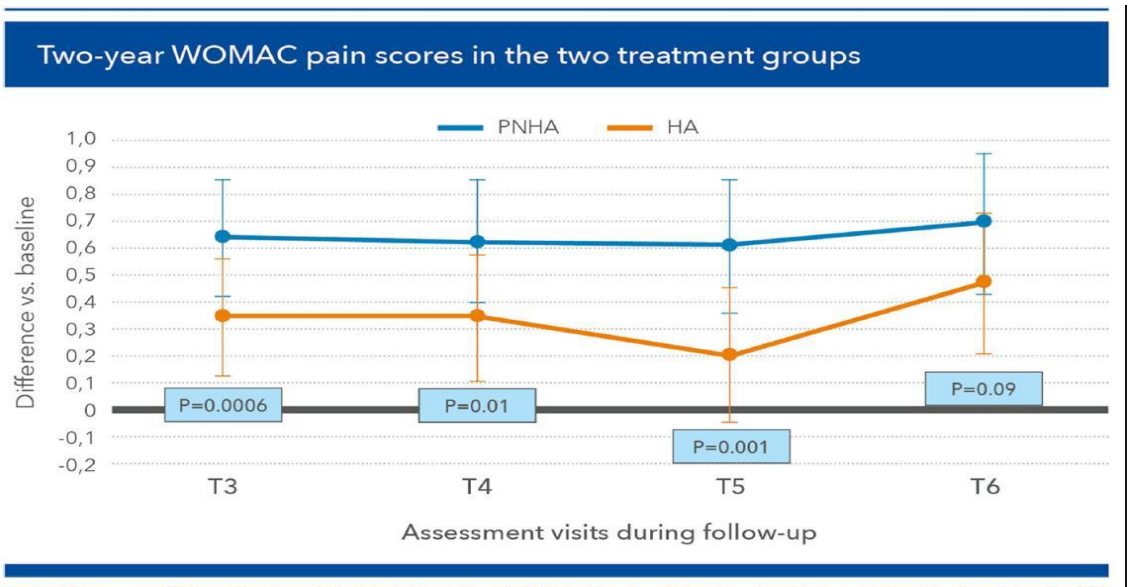


Fig. 3 Differences in Western Ontario and McMaster Universities (WOMAC) pain scores (primary endpoint; mean ± SD) vs baseline during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline)

Distribution (%) of patients in the 2 treatment groups according to the disease severity

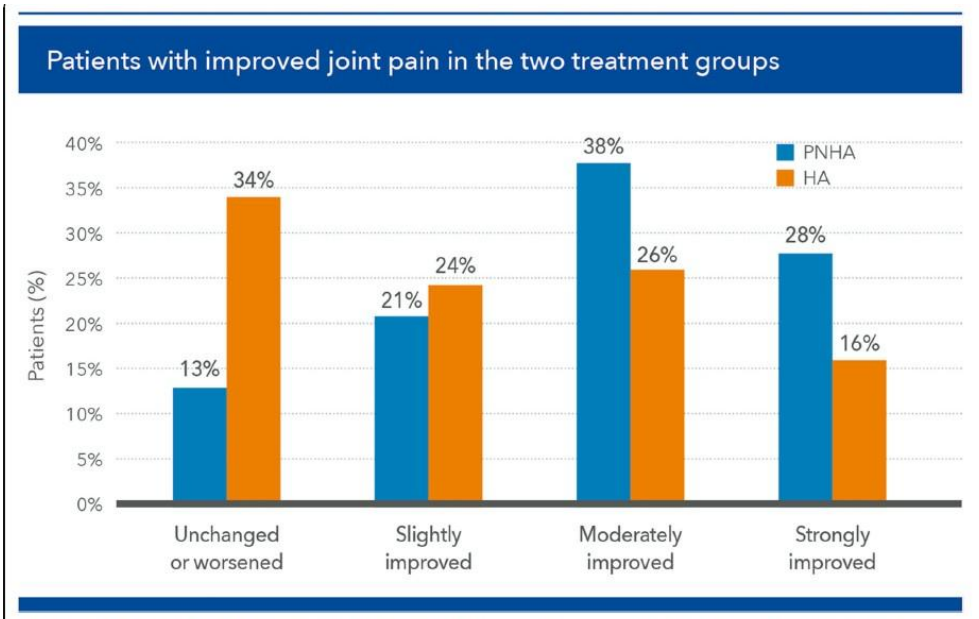


Fig. 7 Percent of patients in the fixed combination (PNHA) and hyaluronic acid (HA) treatment groups reporting improvement in Knee Society Score (KSS) pain scores during the [T3] (2 months) to [T6] (24 months) follow-up period

Conclusions

The two-year double-blind study outcomes, confirmed natural-origin, highly purified polynucleotides (**PN-HPT™) as agents with long-acting viscosupplementation and persistent protective activity in chondrocytes**, **and** a **valuable complement to HA for the relief of pain and functional symptoms in OA**. The indirect PN-HPT™ supporting activity on connective tissues suggested by in-vitro studies, including on chondrocytes and the joint cartilage, might be especially of value as the basis of the likely in-vivo synergy between the two viscoelastic agents[22].

Polynucleotides Positioning according to FMSI (Italian Sports Medicine Federation) Guidelines[23]:

- **PN-HPT** seem to have a **faster response in terms of pain at rest and in motion than HA**
- **PN-HPT could be a therapeutic option in OA non responsive to HA**

Medicina dello Sport, Sept. 73(3):473-502; 2020

Condrotide HA at a glance

- Represents an **innovative strategy** in the treatment of OA
- Offers a better alternative to HA-viscosupplementation
- Acts as "**Symptomatic Slow-Acting"** and **"Disease Modifying"** for OA, with confirmed long-term **efficacy** (up to 24 months)
- **Valid alternative to corticosteroids, NSAIDs** that due to their proven side effects, have to be used for short periods or are contraindicated in many patients
- **Evidence-based efficacy data** in different OA joints (knee, shoulder, hip, ankle) and different therapeutic areas (wound healing, dermatology and aesthetic medicine)
- **Pure and highly concentrated PN** provided by **Mastelli's know-how, as well as** top-standard biotechnologies

4.4 Real world evidence data

Real World Evidence (RWE) is clinical evidence generated from observational data on the real-world use of medical devices in day by day clinical practice. It differs from evidence produced through formal clinical trials because RWE relates to experience

with a device in all settings and in all types of patients/users normally exposed to the device, whereas patients enrolled in randomized clinical trials represent a selected population, according to specific inclusion and exclusion criteria, as for study design.

RWE has become increasingly important following the enactment of the Medical Device Regulation MDR 2017/745, fully implemented from May 2021. **The MDR introduces enhanced requirements for Post-Market Clinical Follow-up (PMCF) of all medical devices, requiring that data be produced continually and throughout the entire lifetime of the device.**

In order to meet these requirements, **medical device manufacturers and distributors need to develop and implement a Clinical Evidence Generation system** that produces RWE on device safety and performance while remaining in compliance with all relevant rules, regulations and wider legislation.

Mastelli S.r.l, in cooperation with its commercial partners, implements RWE studies to comply with requirements for PMCF. **These studies:**

- **are drawn from a** study population that represents the entire **population normally exposed to the device**
- **are non-comparative**, focusing only on the performance of the subject device rather than making comparisons to something else
- **seek to confirm** or refute that the device meets **safety and performance objectives** “out there in the real world”.
- have **no** upper **limit on recruitment number**

- **run** indefinitely, aiming **to cover the "entire lifetime of the device**"
- are conducted **at all types of clinical site**, not just specialist units

CHAPTER 5

CONDROTIDE HA SAFETY AND CLINICAL TOLERANCE DATA

5.1 Safety data from clinical trials

- ***Vanelli study safety data*** (see paragraph 4.3). This study was conducted on 30 patients with knee OA treated with PN-HPT (5 injections) versus HA (Sinovial). **No serious adverse events were reported during the study**; the intra-articular administration of the two products did not cause any systemic undesired event in any patient; only one patient developed mild joint pain after the last PN-HPT injection, which subsided within 1 h.
- ***Giarratana study safety data*** (see paragraph 4.3). This study was conducted on 36 patients with knee OA treated with PN-HPT (3 injections) versus HA (Hyalubrix). The authors of the study concluded: … **"no significant adverse events connected to the employment of both products were reported".**

- ***Saggini study safety data*** (see paragraph 4.3) on 80 patients with rotator cuff syndrome for incomplete lesion of the supraspinatus treated with PN-HPT (4 injections sessions) or Sinovial One, no adverse events were observed.
- ***Migliore study safety data*** (see paragraph 4.3) on 43 hip OA patients treated with PN-HPT (1 injection). The authors observed that: …. "**Neither the patients nor** the attending **physicians reported systemic or severe local side effects**. A **few minor and transient local pains or burning sensations** substantiated all reported side effects.."
- ***Dallari study safety data*** (see paragraph 4.3) on 49 knee OA patients treated with Plinart HA (PN-HPT+HA) for a total of 3 injections and a 12-month follow-up. The authors didn't observe significant adverse events.
- Neither infiltrative treatment was associated with short-term complications or long-term side effects of any clinical significance were observed in the same patients followed in a 24-month follow up (**Stagni study safety data**, par. 4.3)

5.2 Post-Marketing Surveillance Data

To fulfill the requirements of post-marketing surveillance, **Mastelli S.r.l collected and processed safety data on Plinart HA, starting from marketing in Italy up to 2018**. Analyzed events are related to:

- clinical and laboratory adverse events in patients
- medical literature safety analysis
- clinical trials analysis

In the up-to-date report (Code document “DDM22”) it is claimed that “to Manufacturer’s knowledge there are no evidences of reported issues related to the product. **Manufacturer considers that post-marketing surveillance data confirm the favorable risk-benefit product clinical profile** and these data are matching the content of current product information leaflet (IFU)”.

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