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INTRA-ARTICULAR INJECTIONS OF PLATELET-RICH PLASMA COMBINED WITH HYALURONIC ACID VERSUS HYALURONIC ACID ALONE IN TREATMENT OF KNEE OSTEOARTHRITIS.

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ABSTRACT

Osteoarthritis (OA) is a complex disease caused mainly by inflammatory mediators. The last non-operative treatment modality, when other conservative treatments are ineffective is intra-articular (IA) injections with corticosteroids, viscosupplements or blood-derived products. **Purpose:** The aim of this study was to compare the efficacy of Cellular Matrix-PRP-HA (CM-PRP-HA), a combination of autologous platelet-rich plasma (PRP) and hyaluronic acid (HA) prepared using a dedicated medical device, to HA alone in IA injections for treatment of osteoarthritic knee. **Methods**: The study involved 100 symptomatic patients with knee cartilage degenerative lesions (Kellgren Lawrence scale grade 0 to III). Fifty patients were treated with IA injections of autologous CM-PRP-HA combination that was repeated 3 times with 3 weeks interval, while the other 50 patients were treated with IA HA injections. All patients were clinically evaluated pretreatment (basal) and at 2,6 and 12-month follow up visits by using **Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Results:** At each follow up visit, both groups showed a highly significant improvement when compared with the basal assessment and the same results were obtained upon comparing 12- month follow up visit with the 2- month one. The infra-group comparison showed, at each follow up evaluation, a significantly higher improvement for the group treated with CM-PRP-HA in respect to HA alone. **Conclusion:** IA injections of CM-PRP-HA showed more efficacy than HA injections in reducing pain and symptoms of mild to moderate knee degenerative osteoarthritis.

KEYWORDS: knee osteoarthritis, autologous PRP and hyaluronic acid.

1. INTRODUCTION

Osteoarthritis (OA) is a major source of disability owing to pain and loss of function. It is the most common form of joint disease and among the top 10 causes of disability worldwide. OA arises as a major public health problem and an important financial burden for the global economy.[1] The Osteoarthritis Research Society International (OARSI) definition of OA is: a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness.[2]

The prevalence of OA is increasing and its consequences are having a significant impact on society. Study done in KSA in Qassim found that 766 cases (13%) of clinical OA of the knee in the 5,894 adult population and The prevalence increased with increasing age reaching 30.8% in those aged 46-55 years and 60.6% in the age group 66-75 years.[3] The lifetime risk of developing symptomatic knee OA is estimated to be ~45% (40% in men and 47% in women) based upon Johnston County Osteoarthritis Project data, with risks increasing to 60.5% among persons who are obese, which is approximately double the risk of those who are of normal weight or are underweight.[4]

The initial management in OA usually begins with analgesia and anti-inflammatory agent.[5] Topical agents and Intra-articular (IA) injections of corticosteroids, as indicated by a few studies, are usually of short-term benefit for pain and improving the function in mild osteoarthritis.[6] The IA injection of Hyaluronic Acid

(HA) (viscosupplementation) is thought to restore normal viscoelastic properties of the pathologically altered synovial fluid.[7] Also HA has disease modifying effects by reduction of synovial inflammation[8], protection against cartilage erosion[9] and promotion of IA HA production.[10]

Platelet-rich plasma (PRP) is a portion of the plasma fraction of autologous blood having a platelet concentration above baseline.[11] PRP injections have a great acceptance in orthopedics, cosmetics, maxillofacial surgery, cardiovascular surgery and urology.[12]

Platelet contains many chemokines and growth factors that can initiate all wound healing process. These growth factors are: platelet-derived growth factors (a,b), transforming growth factor (TGF)-β, vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor, Connective tissue growth factor and insulin like growth factor-1.[13] PRP also includes plasma proteins known to act as cell adhesion molecules: Fibrin, fibronectin and vitronectin.[14] Platelet released growth factors can stimulate repair of damaged cartilage[15] and even protect the cartilage and lubricate the joint by regulating endogenous hyaluronic acid (HA) synthesis.[16] All these growth factors act on their target cells; which include mesenchymal stem cells from joint tissues such as the synovium or the Hoffa fat[17] (promoting both migration along chemokines and growth factors concentration gradient and chondrogenic differentiation), chondrocytes (synthesis of aggrecan and collagen type II), osteoblasts, fibroblasts, endothelial cells and epidermal cells causing direct cellular proliferation, preventing chondrocyte and MSC apoptosis, matrix formation, osteoid production and collagen synthesis; thus provoking tissue repair and regeneration.[18]

PRP also contains a variety of plasma proteins, which are known to be critical components in the healing mechanism of connective tissues.[19] The most important among them are fibrinogen and other clotting factors, as when activated they form a provisional fibrin that can bind several plasma proteins including vitronectin, fibronectin, Von Willebrand factor (vWF) and thrombospondin. Fibronectin is known as a major factor in human serum to recruit subchondral progenitor cells.[20] Additionally, these proteins within the fibrin bind growth factors and form molecular complexes that can dramatically enhance their potency. Platelets aggregate along with fibrin fibers can form a three-dimensional scaffold that can act as a reservoir of growth factors exerting favorable effects on cells and for helping cells adhere, migrate and proliferate.[21,22] These benefits of the PRP fibrin matrix have been clinically well-known in maxillofacial surgery and chronic wound repair.[23,24] PRP scaffold may mimic the initial stage of wound healing and tissue repair that articular cartilage cannot initiate by itself as it is avascular tissue composed of post-mitotic cells incapable of proliferation.[25]

Numerous anti-inflammatory cytokines may be released from platelets, including IL-1 receptor antagonist (IL-1ra) soluble tumor necrosis factor (TNF) receptor (sTNF-R) I and II, IL-4, IL-10, IL-13, and interferon γ.[26] IL-1ra blocks the receptors of IL-1 and inhibits its bioactivity. Signal transduction of TNFα is prevented when sTNF-RI and sTNF-RII bind to free TNFα.[27] IL-4, IL-10 and IL-13 can act through both increases IL-1ra production and reduced TNFα-induced prostaglandin E2 production.[28,29] IL-18-binding protein production is induced by Interferon γ thus inhibiting IL-18.[30] Pro-inflammatory cytokines are also released from platelets, such as IL-1α, IL-1β, TNFα, IL-6, IL-8, IL-17 and IL-18; however their concentrations are much lower than those of the anti-inflammatory counterparts.[26] So the upper hand in PRP cytokines is for anti-inflammatory cytokines suggesting that PRP may have a crucial role in suppression of inflammation in osteoarthritis (OA), thereby protecting cartilage and reducing pain.[25]

Collectively, IA PRP can interfere with the catabolic microenvironment in OA joints, modulating the inflammatory response, inducing cell migration and proliferation and regulating angiogenesis and cell differentiation.

2. Subjects and methods

2.1 Study design: Randomized clinical trial**.**

2.2 Study area: King Abdul Aziz Hospital at Taif City.

2.3 Patient Selection: questionnaires replied by One hundred patients subjected to this study, 50 of them treated with hyaluronic acid (HA) intra-articular knee injection and the other 50 patients treated with Platelet Rich Plasma - Hyaluronic acid (Cellular Matrix-PRP-HA) intra-articular knee injection. The internal review board and hospital's ethics committee had approved prospective comparative study of this clinical experimentation and informed consent obtained in all patients. The Inclusion criteria for patient selection were history of knee pain or swelling not improving with medications and physiotherapy for at least 4 months and presence of degenerative changes in the joint (Kellgren – Lawrence scale grade 0 to III)[31] in the plain radiographs or magnetic resonance images (MRI). In all patients, plain radiographs done to determine the OA grade. For Kellgren-Lawrence grade 0 patients, MRI done to determine the presence chondral lesions. Patients without evidence of cartilage changes on MRI were excluded from the study. Symptoms were due to the degenerative knee condition and not related directly to previous trauma.

The Exclusion criteria included systemic disorders such as diabetes mellitus, rheumatoid arthritis, bleeding disorders (coagulopathies), severe cardiovascular diseases, infections, the use of immunosuppressive drugs, patients receiving anticoagulants, use of nonsteroidal anti-inflammatory drugs in the last 5 days before blood donation and patients with hemoglobin

(g/dl) values of less than 11 and platelet values of less than 150,000/mm^3. Patients who met these criteria were randomized into a PRP/ HA group or a HA group.

2.4 CM-PRP-HA preparation and IA injection

Cellular Matrix-PRP-HA (Regen lab cellular matrix/A-CP-HA)[32] is a synergistic association of autologous platelet rich plasma and hyaluronic acid. It is prepared by adding 4 ml of autologous venous blood to a closed tube containing 2ml of natural, non-crosslinked, at a concentration of 20 mg/ml (40 mg total) in addition to the thixotropic cell-separation gel and the sodium citrate anticoagulant solution. One step closed system centrifugation of this mixture at 3100 rpm for 5 minutes is used to separate erythrocytes and produce 4 ml of PRP-HA mixture (figure 1). The skin was sterilely dressed, and the Intra articular injection was performed using the 4 ml of PRP-HA mixture through a classic Lateral or medial approaches with a 22-gauge needle. At the end of the procedure, the patient was encouraged to bend and extend the knee a few times to allow the PRP-HA mixture to distribute itself all over the joint. This process was repeated 3 times with 3 weeks interval.

2.5 Regen lab PRP properties: platelet recovery superior to 80% and a concentration factor of 1.6-fold times over the baseline. White blood cells are strongly reduced (> 85% depletion).

2.6 Post-procedure follow-up and evaluation

Patients were prospectively, clinically evaluated before the treatment and at 2, 6 and 12 months follow-up visits by WOMAC score.[33] After each injection, the patients were sent home with instructions on limiting the use of the leg and to not use nonsteroidal medication but to use cold therapy for pain for at least 24 hours. During the injection cycle, rest or mild activities (such as exercise bike or mild exercises in a pool) were indicated, and subsequently, a gradual resumption of normal sport or recreational activities was allowed as tolerated in all the treatment groups. Adverse events and patient satisfaction were also recorded.

WOMAC questionnaire is a tool used widely in studies of arthritis that includes five items for pain, two items for stiffness, and 17 items for assessing functional limitation. Each question is scored from 0 to 5 with fewer scores indicating less pain and better functional status.[33]

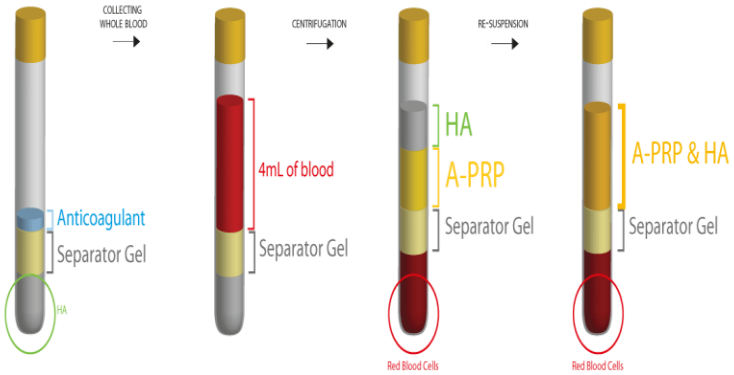

Figure 1: Preparation of Cellular Matrix-PRP-HA

2.7 Statistical Analysis

Data was collected and entered to the computer using SPSS (Statistical Package for Social Science version 22) program for statistical analysis. Suitable tests of significance were used according to the type of data and a P value less than 0.05 were considered statistically significant.

III. RESULTS

3.1 Distribution of study participants according to demographic criteria: As for all the 100 patients; age ranged from 45 to 75 years old with Mean± SD (59.34± 6.81). Male: female was 41:59. As regard occupation; 55 patients were Unemployed, 25 employer and 20 retired patients. As for the same items in the two groups. Table (1) shows these data distribution among the two groups with statistically non-significant differences between them as regard age, sex, occupation and disease degree.

3.2 Distribution of study participants according to type and symptom of OA: pain was the most frequent complain (38/100), followed by joint stiffness (22/100) and effusion (19/100) and combined symptoms (21/100). Mild OA compromised (48/100), while Moderate OA was (52/100).

3.4 Basal, 2 m, 6m and 12 m assessment of HA and CM-PRP-HA and their comparison

At each follow up visit, both groups showed a highly significant improvement when compared with the basal assessment and the same results were obtained upon comparing 12- month follow up visit with the 2- month one. The infra-group comparison showed, at each follow up evaluation, a significantly higher improvement for the group treated with CM-PRP-HA in respect to HA alone.

Table (1): Distribution of study participants according to demographic criteria

	CM-PRP-HA	HA	(P value)
Sex **1-Male** **2-Female**	 24 (48 %) 26 (52%)	 17(34%) 33 (66%)	0.2223
Age/ year **1- Minimum** **2- Max** **Mean**±SD	 46 75 58.26±7.131	 45 73 60.42±6.36	0.1132
Type **Mild** **Moderate**	 25(50.0%) 25(50.0%)	 23 (46.0%) 27 (54.0%)	0.814
Occupations **1- Employment** **2-Unemployment** **3- Retired**	 15 (30%) 24 (48 %) 11 (22%)	 10 (20 %) 31 (62.0%) 9 (18.0 %)	 0.3558 0.2276 0.8031

Table (2): Basal, 2 m, 6m and 12 m assessment of HA and CM-PRP-HA:

Pain	HA Mean±SD	HA	CM-PRP-HA Mean±SD	CM-PRP-HA	CM-PRP-HA versus HA
Basal	6.319±10	Basal vs 2 mon t-value: 13.7 P < 0.0001	6.46 ± 1.55	Basal vs 2 mon t-value: 16.8 P < 0.0001	Basal vs Basal t-value: 0.5 P = 0.6307
2 mon	4.61±1.18	Basal vs 6 mon t-value: 10.9 P < 0.0001	2.64 ± 1.22	Basal vs 6 mon t-value: 14.5 P < 0.0001	2 mon vs 2 mon t-value: 16.5 P < 0.0001
6 mon	4.91±1.38	Basal vs 12 mon t-value: 5.4 P < 0.0001	3.26 ± 1.39	Basal vs 12 mon t-value: 12.1 P < 0.0001	6 mon vs 6 mon t-value: 5.96 P < 0.0001
12 mon	5.97±1.07	2 mon vs12 mon t-value: 9.99 P < 0.0001	5.00 ± 1.14	2 mon vs 12 mon t-value: 5.9 P < 0.0001	12 mon vs 12 mon t-value: 4.4 P < 0.0001

IV. DISCUSSION

Osteoarthritis (OA) represents a major cause of knee disability involving cartilage damage related to an inadequate healing response in the inflammatory milieu.[34] Current non-surgical modalities of treatment include weight reduction, physiotherapy, analgesia, non-steroidal anti-inflammatory drugs and intra-articular injections; such as hyaluronic acid (HA), corticosteroids, or Ozone; all are used for reducing symptoms and improving joint function.[35]

In this study both groups showed a highly significant improvement, however CM-PRP-HA group showed a significantly higher improvement compared to HA group.

There are a lot of studies that compare PRP alone versus HA alone and most of them proved that PRP is superior to HA. Few studies are similar to this study in studding combined PRP/HA against HA alone. So, at the beginning of this discussion, the use of intra-articular injection of PRP in mild to moderate osteoarthritic knee will be discussed, then comes the part that discusses using combined PRP/HA in such cases.

As regard assessing the use of intra-articular injections of PRP against HA; Raeissadat et al. conducted a non-placebo-controlled randomized clinical trial that involved 160 patients affected by knee OA, grade 1– 4 of Kellgren–Lawrence scale, where the PRP group (n = 87) was treated with two intra-articular injections of leukocyte-rich PRP at 4-week interval and in the HA group (n = 73) treated with three doses of intra-articular injection of HA at 1-week interval were applied. Platelet concentrations were 5.2 ± 1.50 times and 4.8 ± 1.80 times the baseline values in the first and second preparations, respectively. They had noted that PRP displayed more effective improvements in WOMAC score and patients' functions than HA at 12 months post injection.[36]

Montañez-Heredia et al. had also reached to the similar results, as they conducted a double-blind randomized controlled clinical trial. Pain and functional improvements were assessed pre- and post-treatment twice at 3 & 6 months follow-up using the Visual Analogue Scale (VAS); the Knee and Osteoarthritis Outcome System (KOOS) scale and the European Quality of Life scale (EUROQOL). The PRP they used met requirements of commercials platelet concentrate used for transfusion therapy as regards concentration of

growth factors content, RBC and WBC count. They found that both groups showed pain reduction at 6 months and the PRP group score improved by at least 50% from their initial value. However they noticed that PRP was more effective in patients with lower osteoarthritis grades.[37]

Cole et al., 2015 study demonstrated the superiority of intra-articular injection of PRP over HA in patients with mild to moderate knee OA at both clinical and biological level. At the clinical level, there was a significant improvement in WOMAC pain score. At the biological level, there was a trend toward a decrease in tumor necrosis factor-a and interleukin-1b in synovial fluid samples after intra-articular injection of PRP, which suggest that the anti-inflammatory properties of PRP may contribute to an improvement of symptoms. They utilized a low-leukocyte platelet preparation by a single-spin that concentrates platelets and separates red blood cells as well as white blood cells (WBCs) from the treatment product with platelet concentration 1.73 ± 0.05 when compared with whole blood.[38]

At gene expression level, Sundman et al, proved that PRP can stimulate endogenous HA production and decrease cartilage catabolism by harvesting synovium and cartilage from patients undergoing total knee arthroplasty and co-culturing in media with PRP or HA. They reported that both PRP and HA treatments of OA joint tissues result in decreased cartilage catabolism, but PRP treatment also resulted in a significant reduction of matrix metalloproteinase-13 expression, an increase in hyaluronan synthase-2 expression in synoviocytes, and an increase in cartilage synthetic activity compared with HA. They also demonstrated that both PRP and HA showed similar effects in the suppression of inflammatory mediators concentration and expression of their genes in synoviocytes and cartilage.[39]

A recent systematic review and meta-analysis study done by Shen et al. included fourteen randomized controlled trials comprising 1423 participants assessed the temporal effect of platelet-rich plasma on pain and physical function in the treatment of knee osteoarthritis. The control included saline placebo, HA, ozone and corticosteroids. They displayed that PRP injections significantly reduced total WOMAC scores, WOMAC pain and physical function subscores at 3, 6 and 12 months follow-up ($p = 0.02$, 0.004, <0.001, respectively); nonetheless, PRP did not significantly increased the risk of post-injection adverse events.[40]

Another systematic review and meta-analysis study done by Kanchanatawan et al. to compare not only the clinical outcomes of osteoarthritis indices (WOMAC and Lequesne scores) but also the adverse events in the treatment of OA of the knee with PRP versus HA or placebo. They had concluded that PRP injection has significantly improved functional outcomes when compared to HA and placebo without having a statistically significant difference in adverse events.[41]

As regards the PRP preparation in this study; Cellular Matrix-PRP-HA tube prepares 4 ml of autologous platelet rich plasma mixed with HA; with a platelet recovery superior to 80% and a concentration factor of 1.6-fold that of blood which is optimum for healing. Some studies demonstrated that concentrations of platelets 1 to 3 times over the baseline show more robust healing rates than those with concentrations of 3 to 8 times the baseline.[42] On the contrary; too high platelet concentrations may actually have negative effects on osteoblast activity, probably due to unwanted inhibitory and cytotoxic effects of growth factors at such high concentrations.[43] Similarly; Weibrich G. et al. demonstrated that platelet concentration over 2.5-fold, resulted in a reduction in proliferation and a suboptimal effect on osteoblast function.[44]

White blood cells are strongly reduced (> 85% depletion) in Cellular Matrix-PRP-HA. The role of white blood cells (WBC) in healing is controversial. Granulocytes, and more specifically neutrophils, are associated with the inflammatory response as they release a large variety of highly active antimicrobial substances and proteases that can cause severe damage to the tissue, delay healing rates and increase the risk of scarring.[45] The few white blood cells still present are mostly mononuclear cells (lymphocytes and monocytes). These two cell types are also involved in the immune response but have been shown to support the healing process.[46,47]

Viscosupplementation with HA in knee OA has been approved by the FDA[48] and is recommended by OARSI for non-severe OA.[49] Compared to HA, the use of PRP in the treatment of knee OA is more recent; however it showed advantages over HA. Separately; HA and PRP are beneficial for joint cells although they function through different mechanisms. Therefore, the combined use of PRP and HA may assume that their advantages might be additive when both products are injected in the knee OA. That can be noticed in Sundman et al.[39] study, as it showed that both PRP and HA have anti-inflammatory effect but in two different ways, as both decreased TNF-α production but only HA that caused IL-6 reduction. The study also noted that PRP treatment resulted in a significant reduction of matrix metalloproteinase-13 expression (catabolic enzyme) and an increase in hyaluronan synthase-2 expression in synoviocytes leading to increase in cartilage synthetic activity; while HA did not show such results.

Anitua et al. *in vitro* study[50] demonstrated the effect of PRP with HA on human tendon cells and synovial fibroblasts migration. Both PRP and HA stimulated the migration of fibroblasts, but this effect was more prominent when both were combined. Obviously, PRP improves the biological properties of HA especially migratory effect, that may be by enhancing both CD44

and receptor for HA-mediated motility (CD168) expression, which are needed for HA migratory signal transduction. HA-CD44 binding is also involved in the resolution of inflammation. Besides, both CD44 and CD168 are involved in the regulation of growth factor signaling.[51,52]

Recently, Russo et al. study demonstrated that PRP addition is not detrimental to the viscosupplementation effect of HA. They also found that *in vitro* culture of human chondrocytes showed significantly higher proliferation rate and glycosaminoglycan content when cultured in the media containing PRP blend with Sinovial 3.2% compared to the cultures with HA alone.[53] It is clear from their work that type, concentration and molecular weight of HA affect net result of the blend with PRP. As formulations with HA concentration below 1% displayed significant drop of viscoelastic properties upon mixing with PRP. Also other types and other concentrations of HA (Sinovial, 0.8%, Sinovial, 0.8% and Hyalubrix 1.5%) did not cause chondrocyte proliferation in the *in vitro* culture.

Chen. et al. investigated the therapeutic effects of HA and PRP and their combination on *in vitro* culture of chondrocyte from OA patients.[54] They demonstrated that the combination of HA and PRP promoted cartilage regeneration and inhibited OA inflammation via decreasing expressions of inflammatory genes and OA pathology-related chemokines and cytokines genes. Moreover, the cartilaginous ECM could be retrieved from inflammation-induced degradation by retrieving pro-inflammatory cytokines-reduced articular chondrocytes proliferation. So, they inferred that the intra-articular injection of HA+PRP could strongly rescue the meniscus tear and cartilage breakdown and then decrease OA-related immune cells and that the combination of HA+PRP can synergistically promote cartilage regeneration and inhibit OA inflammation.

As regard the *in vivo* studies; Abate et al. treated 40 patients with mild-to-moderate knee osteoarthritis with weekly intra-articular injection of 2 ml of hyaluronic acid combined with 2 ml of platelet-rich plasma for 3 weeks. Clinical and functional assessments were performed at 1, 3 and 6 months and the efficacy of this combination was compared retrospectively with those of a cohort of patients treated with platelet-rich plasma only. The intra-group comparison showed a significant improvement while the infra-group comparison did not show any significant difference. They concluded that The combination of platelet-rich plasma and hyaluronic acid has the same efficacy of platelet-rich plasma only, administered in higher volume. They also deduced that hyaluronic acid works synergistically and improves the activity of several molecules contained in platelet-rich plasma.[55]

Another recent randomized Controlled study done by Dallari et al. done on a total of 111 patients with hip OA, as they were randomly assigned to 3 groups and received 3 weekly injections of either PRP (44 patients), PRP+HA (31 patients), or HA (36 patients). All patients were assessed by the VAS, Harris Hip Score and WOMAC score at 2, 6 and 12 months after treatment. Their results indicated that intra-articular PRP injections offer a significant clinical improvement in patients with hip OA without relevant side effects. On the contrary, they observed that the addition of PRP+HA did not lead to a significant improvement in pain symptoms.[56] The difference in this study results from our results may be due to using different PRP/HA preparation and using it for hip joint instead of knee joint.

V. CONCLUSIONS

Both PRP and HA have been extensively used to improve lubrication, modulate inflammation and modify the joint catabolic micro-environment, aiming not only for reducing clinical symptoms, but also interfere with OA progression. In the present study, intra-articular PRP/HA injections (Cellular Matrix-PRP-HA) are shown to be more effective in the treatment of knee OA as regard pain relief and function improvement at 2, 6 and 12 months follow-ups, compared with HA alone. The effectiveness of combined PRP/HA for knee OA should be further verified by larger studies to evaluate the proper hyaluronic acid characters that ensure the additive advantages of both compounds .

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