Article

Not peer-reviewed version

Evaluating Synergistic Effects of Hyaluronic Acid, Human Umbilical Cord-Derived Mesenchymal Stem Cells, and Growth Hormones in Knee Osteoarthritis: A Multi-Arm Randomized Trials

Ismail Hadisoebroto Dilogo *, Anissa Feby Canintika, Bernadus Riyan Hartanto, Jacub Pandelaki, Irsa Gagah Himantoko

Posted Date: 14 August 2024

doi: 10.20944/preprints202408.1003.v1

Keywords: knee osteoarthritis; mesenchymal stem cells; hyaluronic acid; growth hormones; cartilage regeneration

 doi:10.20944/preprints202408.1003.v1

Article

Evaluating Synergistic Effects of Hyaluronic Acid, Human Umbilical Cord-Derived Mesenchymal Stem Cells, and Growth Hormones in Knee Osteoarthritis: A Multi-Arm Randomized Trials

Ismail Hadisoebroto Dilogo [1,2,3,*], Anissa Feby Canintika [1], Bernadus Riyan Hartanto [1], Jacub Pandelaki [4] and Irsa Gagah Himantoko [1]

[1] Department of Orthopaedic and Traumatology, Dr. Cipto Mangunkusumo General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2] Stem Cell Medical Technology Integrated Service Unit, Cipto Mangunkusumo Central Hospital, Faculty of Medicine, Universitas Indonesia, CMU 2 Building 5th Floor, Jl. Diponegoro 71, Jakarta Pusat, Indonesia
[3] Stem Cell and Tissue Engineering Research Center, IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[4] Department of Radiology, Cipto Mangunkusumo General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[5] Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia
* Correspondence: ismailortho@gmail.com

Abstract: Background: Knee osteoarthritis (OA) significantly affects quality of life and imposes economic burdens due to its prevalence and the disability it causes. The efficacy of current treatments is limited to alleviate the symptoms and not for regenerative purposes. This study aims to evaluate the efficacy and safety of combining hyaluronic acid (HA), human umbilical cord mesenchymal stem cells (hUC-MSCs), and synthetic human growth hormone (somatotropin) in the treatment of knee OA, assessing pain relief, functional improvement, and cartilage regeneration. **Methods**: A four-arm, double-blind randomized trial was conducted with 51 knees from 28 subjects, aged ≥ 50 with primary knee OA. Treatments involved are HA alone, HA with hUC-MSCs, HA with somatotropin, and a combination of all three. Efficacy was measured through the International Knee Documentation Committee (IKDC) score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and visual analog score (VAS) along with MRI T2 mapping of cartilage were taken on pre-implantation, 6th and 12th month. **Results**: All treatment arms showed improvement in VAS and WOMAC scores over 12 months, suggesting some pain relief and functional improvement. However, MRI T2 mapping showed no significant cartilage regeneration across the groups. **Conclusion**: While combined use of HA, hUC-MSCs, and somatotropin improved symptoms of knee OA, it did not enhance cartilage regeneration significantly. The study highlights the potential of these combinations for symptom management but underscores the need for further research to optimize these therapies for regenerative outcomes.

Keywords: knee osteoarthritis; mesenchymal stem cells; hyaluronic acid; growth hormones; cartilage regeneration

1. Introduction

Knee osteoarthritis imposes a significant burden on patients, caregivers, and payers owing to its prevalence, impact on quality of life, and economic implications. Patients with knee osteoarthritis often experience pain that can lead to lifestyle modifications [1]. This pain not only affects their daily activities but also contributes to a decrease in their overall quality of life [2]. The burden is further exacerbated by the increasing prevalence of knee OA, attributed to factors such as the global rise in

 doi:10.20944/preprints202408.1003.v1

obesity and an aging population [3]. Additionally, knee OA is highlighted as a major cause of disability, especially among older individuals, placing a strain on healthcare resources [4].

Despite the burden imposed by knee OA, current treatment options have limitations. The management of knee OA often involves a combination of pharmacological and nonpharmacological interventions. However, the effectiveness of these treatments can vary, and some patients may not achieve adequate pain relief or functional improvement [5]. Furthermore, the increasing prevalence of knee OA poses a challenge to healthcare systems, necessitating the development of more cost-effective and sustainable management strategies.

Hyaluronic acid (HA) has been shown to be safe and effective for knee osteoarthritis, offering long-lasting improvement in clinical parameters. While some studies found that combining hyaluronic acid with corticosteroids enhances pain control, other research indicates that hyaluronic acid alone is a viable option for long-term pain relief and improved function [6,7]. Aside from hyaluronic acid, synthetic human growth hormone has been a sought-after active ingredient for knee osteoarthritis. A clinical trial by Rahimzadeh et al. found that adding growth hormone (Somatropin) to platelet-rich plasma could be effective. Additionally, a rabbit model study by Palmieri et al. indicated that combining growth hormone with hyaluronic acid resulted in better outcomes than hyaluronic acid alone, suggesting enhanced therapeutic potential [7,8]

Mesenchymal stem cells have gained popularity because of their ease of harvesting, safety, and potential to differentiate into cartilage tissue [9]. Clinical trials have demonstrated the safety and effectiveness of MSCs in promoting cartilage regeneration and alleviating symptoms of knee OA [10]. Additionally, studies have shown that stem cell-derived extracellular vesicles (EVs) can be effective in treating joint injuries and OA, offering regenerative effects and therapeutic benefits [11]. Despite the promising outcomes reported in various studies, there are still limitations and challenges associated with stem cell therapy for OA. The heterogeneity of cell entities and concomitant procedures in clinical studies have led to unclear evidence regarding the efficacy of MSCs in knee OA [12]. While preclinical and clinical trials have shown initial evidence of efficacy and safety in using MSC therapies for knee OA, more robust randomized controlled trials are needed to establish the definitive efficacy and efficiency of these treatments [13].

2. Materials and Methods

This four-arm, double-blind, randomized study was conducted from November 2019 to October 2023 at Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. The subjects included in this study were patients aged ≥ 50 years with a diagnosis of primary OA of one or both knees based on the American College of Rheumatology (ACR). Osteoarthritis was diagnosed using historical data, physical examination, and radiographic evidence of joint changes according to Kellgren–Lawrence grades I–IV 6 months prior to the commencement of the research.

The study was registered at ClinicalTrial. gov (reference NCT03800810). After an IRB approval (KET-1149/UN2.FI/ETIK/2019), informed consent was obtained following the most recent version of the Helsinki Declaration. Knee osteoarthritis was classified based on the Kellgren and Lawrence system using typical knee X-ray images taken in standing anteroposterior and horizontal lateral views. One radiologist performed image interpretation and staging separately. The inclusion and exclusion criteria are listed in Table 1.

Table 1. Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion criteria
Primary osteoarthritis in one or both knees	Experienced a knee joint infection or an infection in the skin or soft tissue around the knee.
Radiographic evidence of Kellgren–Lawrence Grade I-II (mild to moderate) knee joint changes	Had a history of cancer
Body mass index (BMI) of below 30 kg/m^2	

 doi:10.20944/preprints202408.1003.v1

2.1. MRI

Knee MRI scans were performed with a GE Optima MR450W (wide bore) 1.5 T (GE Healthcare, GE Healthcare, Waukesha, WI, USA). Scans were further analyzed using Advance Workstation 4.6 (AW 4.6) and processed to achieve a colorized T2 map using CartiGram software (GE Healthcare, Waukesha, WI). The T2 mapping sequence was obtained using the following parameters: coronal orientation, 256×256 matrix, 16×16 cm field of view, 4 mm slice thickness, 1.5 mm slice gap, 62.5 kHz receiver bandwidth, 4 min acquisition time, TR of 1000 ms, TE of 8.3, 16.6, 24.9, 33.2, 41.4, 49.7, 58, 66.3 ms and color range, 25–75 ms.

MRIs was performed initially, and at 6 and 12 months. Knee MRI was performed using a standard technique that included imaging in the axial, coronal, and sagittal planes. In addition, a special cartilage sequence, T1-weighted FS spoiled 3D gradient echo, was used in the axial and sagittal planes. Precise measurements were collected from each compartment at three specific points: anterior, middle, and posterior. Subsequently, the average thickness was computed. Identical sequences and measurement locations were used in follow-up scans.

2.2. Treatment

Prior to receiving the injection, subjects were recruited and subsequently allocated into one of the four treatment groups based on a pre-randomized treatment allocation table. Patient allocated on group 1 received intraarticular hyaluronic acid (HA) (Suplasyn, Mylan) of 20 mg/2ml (1 syringe) per week for three weeks, group 2 received combination of intra-articular human umbilical cord mesenchymal stem cells (hUC-MSCs) with HA in the first week and HA only for the subsequent two weeks, group 3 received combination of somatotropin (Saizen, Merck) of 5.83 mg/1ml (1 syringe) with HA in the first two weeks and HA only on the last weeks, and group 4 received a combination of hUC-MSCs, HA and somatotropin in the first week, HA and somatotropin in the second week, and HA only in the third week.

The subjects diagnosed with knee osteoarthritis received three injections in our trial. During the initial session, participants received an intra-articular injection according to the allocated treatment arm. During the second and third weeks, the individuals received a 2 ml HA injection. The dosage was consistent with that utilized in a caprine model of osteoarthritis in a study conducted by Murphy et al. [14]. hUC-MSCs were obtained using the multiple-harvest explant approach and cultured in a xeno-free mix containing 10% platelet lysate prepared in-house, as detailed in our previous study [15].

The subjects were then monitored on the 1st and 3rd months, followed by assessments every 3 months for up to 1 year. Outcome measures included the International Knee Documentation Committee (IKDC) score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and visual analog score (VAS). T2 mapping of the knee cartilage was conducted before implantation and again at the 6th and 12th month post-implantation.

2.3. Multiple Harvest Method [16]

The umbilical cord from a healthy full-term delivery was dissected and the umbilical artery and vein were removed and cut into small pieces. The pieces were placed individually in 24-well plates and submerged in a small quantity of medium to keep them moist and prevent them from floating. The specimens were then incubated at 37 °C and 5% CO2. Cell proliferation and desiccation were monitored daily. Additional media was supplied as needed, and cultures were harvested when they reached 80–90% confluence. The collected cells were grown again to produce a sufficient amount for administration to patients. The umbilical cord from a healthy full-term delivery was dissected and the umbilical artery and vein were removed and cut into small pieces. The pieces were placed individually in 24-well plates and submerged in a small quantity of medium to keep them moist and prevent them from floating. The specimens were then incubated at 37 °C and 5% CO2. Cell proliferation and desiccation were monitored daily. Additional media was supplied as needed, and

 doi:10.20944/preprints202408.1003.v1

cultures were harvested when they reached 80–90% confluence. The collected cells were grown again to produce a sufficient amount for administration to patients.

2.4. Data Analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows (IBM) version 26. Numerical variables (VAS, WOMAC, IKDC, T2 map) at baseline, and at the 6th and 12th months after implantation, were analyzed for normality using the Shapiro-Wilk test. Subsequently, the data were further analyzed using repeated measures ANOVA in a mixed model. If the repeated measures ANOVA showed a significant result, the data were further analyzed using the Bonferroni post-hoc test to provide direct comparisons of each variable. The T2 map was further analyzed by whether the value was within normal range (40-60) or off the range (below 40 or above 60) throughout the follow up period for each arm. The proportion for each group that are within the range underwent hypothetical testing using Independent Samples Kruskall-Wallis test with Pairwise comparisons.

3. Results

3.1. Characteristics of the Subjects

Twenty-eight subjects were recruited for this study, with a total of 51 knees. Eight (28.6%) patients were male, with a mean age of 53.16 ± 9.11 years. Subjects had a mean BMI of 24.83 ± 2.63 kg/m^2. This study involved 51 knees that were further divided into four groups: arm 1 received HA, Arm 2 received a combination of MSC + HA, Arm 3 received Somatotropin + HA, Arm 4 received MSC + Somatotropin + HA. All patients were categorized as having Kellgren-Lawrence Grade I-II (100%) (Table 2).

Table 2. Characteristics of the subjects.

Variables	Total	Arm 1 (Synovial, Hyaluronic Acid)	Arm 2 (MSC + Hyaluronic Acid)	Arm 3 (Somatotropin + Hyaluronic Acid)	Arm 4 (MSC + Somatotropin + Hyaluronic
No. of patients					
- Patients	28				
- Samples	51	13	12	12	14
Gender					
- Male	8 (28.6%)				
- Female	20 (71.4%)				
Age (years)	53.16 ± 9.11	48.75 ± 6.63	48.29 ± 9.12	56.1 ± 7.03	59.29 ± 10.23
BMI (kg/m^2)	24.83 ± 2.63	25.58 ± 2.38	25.22 ± 4.57	27.02 ± 2.33	26.51 ± 3.67

3.2. Clinical Outcome

Table 3 shows the clinical outcomes assessed from the subjects, consisting of pain and functional assessments using the VAS and WOMAC. Throughout 12 months of observation, all subjects, regardless of treatment allocation, showed a decreasing VAS score, although the changes were statistically insignificant ($p = 0.139$)). The WOMAC score also showed a similar trend of decreasing throughout the observation period for all groups; nevertheless, the changes were not statistically significant. ($p = 0.587$). Therefore, the changes in the VAS and WOMAC scores notably decreased across 12 months, although these changes did not differ statistically.

3.3. Radiographic Outcome

T2 quantitative mapping of the MRI was utilized to assess cartilage changes following intraarticular injection. The results were taken from baseline, 6 months, and 12 months for bilateral knees for both the medial and lateral parts of the knees. Repeated ANOVA tests were used to observe any significant changes found across groups and across time of observation. The results showed no

 doi:10.20944/preprints202408.1003.v1

significant differences, and there was no particular trend across 12 months of observation for each treatment arm T2 mapping data, either between lateral, medial of the left or right knees, showed no significant difference across the treatment arm and time, with p-values of 0.826, 0.802, 0.353, and 0.395 for lateral and medial of the right knees, and lateral and medial of the left knees consecutively. T2 map value for each timepoint and arm were also categorized into whether the value was within the target of normal T2 map value (40-60) or whether it was off (below 40 or above 60). From the analysis on both arms and time point on the T2 map classification showed significant result on the T2 map value of the medial side on the 12th month among four arms (p=0.024).

Table 3. Outcome of the mild osteoarthritis group at baseline, 6th month follow-up, 12th month follow-up.

Outcomes	Time	Mean (SD)				p-value
		Arm 1	Arm 2	Arm 3	Arm 4	
VAS	**Baseline**	4.38 ± 0.74	5.71 ± 1.38	4.89 ± 1.17	5.71 ± 1.7	0.139
	6th month	3.13 ± 0.35	3 ± 0.58	3.33 ± 0.71	3.57 ± 0.98	
	12 month	2.13 ± 0.35	2	2.56 ± 1.01	2.71 ± 0.76	
WOMAC	Baseline	34.62 ± 5.7	30.79 ± 7.46	35.41 ± 10.6	34.96 ± 13.43	
	6th month	21.54 ± 4.88	24.31 ± 4.79	25.17 ± 6.44	23.94 ± 6.89	0.587
	12 month	12.22 ± 3.73	15.15 ± 1.7	18.04 ± 4.23	23.55 ± 20.73	
IKDC	Baseline	45.52 ± 8.7	38.8 ± 12	40.85 ± 9.35	44.82 ± 10.64	0.349
	6th month	58. 4 ± 6.58	47.5 ± 13.11	55.36 ± 6.95	52.59 ± 9.32	
	12 month	70.05 ± 13.1	48.77 ± 16.45	62.51 ± 9.96	58 ± 10. 3	
T2 Map						
Medial	Baseline	59 ± 25. 44	49.42 ± 5	47.8 ± 9.12	68.24 ± 27.67	0.564
	6th month	48.77 ± 11.73	43.81 ± 4.52	43.67 ± 7.04	68.87 ± 39.8	
	12 month	135.79 ± 125.28	137.53 ± 117.25	199.22 ± 115.56	108.77 ± 107.40	
Lateral	Baseline	47.68 ± 9.64	50.24 ± 14.51	50.51 ± 10.62	53.33 ± 12.1	
	6th month	52.71 ± 10.59	46.73 ± 2.40	68.04 ± 29.72	50.55 ± 8.05	0.483
	12 month	43.46 ± 2.24	47.06 ± 5.72	45.62 ± 0.78	50.43 ± 11.89	

Hypothesis test using Mixed Model ANOVA and Bonferonni posthoc.

Time	Variable	Target		p -value
		Within	Off	
T2 Map Baseline Medial	Arm 1	3 (37.5)	5 (62.5)	
	Arm 2	5 (71.4)	2 (28.6)	0.386
	Arm 3	3 (33.3)	6 (66.7)	
	Arm 4	2 (28.6)	5 (71.4)	
T2 Map 6th month Medial	Arm 1	2 (25)	6 (75)	
	Arm 2	4 (57.1)	3 (42.9)	0.314
	Arm 3	2 (22.2)	7 (77.8)	
	Arm 4	4 (57.1)	3 (42.9)	
T2 Map 12th month Medial	Arm 1	2 (25)	6 (75)	
	Arm 2	3 (42.9)	4 (57.1)	0.024*
	Arm 3	0 (0)	9 (100)	
	Arm 4	5 (71.4)	2 (28.6)	
T2 Map Baseline Lateral	Arm 1	4 (50)	4 (50)	
	Arm 2	3 (42.9)	4 (57.1)	0.281
	Arm 3	2 (22.2)	7 (77.8)	
	Arm 4	5 (71.4)	2 (28.6)	
T2 Map 6th month Lateral	Arm 1	4 (50)	4 (50)	
	Arm 2	5 (71.4)	2 (28.6)	0.067
	Arm 3	2 (22.2)	7 (77.8)	
	Arm 4	6 (85.7)	1 (14.3)	
T2 Map 12th month Lateral	Arm 1	4 (50)	4 (50)	
	Arm 2	5 (71.4)	2 (28.6)	0.361
	Arm 3	3 (33.3)	6 (66.7)	
	Arm 4	17 (54.8)	1 (28.6)	

 doi:10.20944/preprints202408.1003.v1

***) Hypothesis Test Using Independent Samples Kruskall-Wallis Test with Pairwise Comparisons**

4. Discussion

The current treatment options for knee osteoarthritis encompass a range of approaches aimed at managing pain, improving function, and addressing the underlying joint pathology. Conventional management strategies focus on pain relief through joint-specific exercises and pharmacological interventions, and in advanced cases, surgical interventions, such as joint replacement surgery [17]. These interventions aim to alleviate symptoms, enhance mobility, and improve the overall quality of life for individuals with knee OA. Intra-articular injections, including corticosteroids and hyaluronic acid, are commonly used to provide localized relief from pain and inflammation in knee A. These injections can help reduce symptoms and improve joint function, particularly in individuals who may not be suitable candidates for surgery or who wish to delay surgical intervention [18].

The literature on the effect of HA for knee OA suggests that intra-articular injections of HA can be beneficial in managing symptoms and improving joint function in patients with knee OA, as it can provide effective pain relief, reduce joint stiffness, and enhance physical function [19]. HA injections are considered an important nonsurgical treatment option for knee OA, along with other interventions such as corticosteroids and platelet-rich plasma injections with better performance in reducing providing long-term pain relief and improving joint function in knee OA, possibly through its anti-inflammatory, anabolic and chondroprotective properties [6,20,21].

Several studies have been conducted on the effect of intra-articular injection of synthetic human growth hormone on knee OA, which suggests promising outcomes in improving knee joint function and reducing symptoms. A comparative double-blind clinical trial by Rahimzadeh demonstrated that adding growth hormone to platelet-rich plasma for intra-articular injection improved the function of the osteoarthritic knee joint in a short period of time, with no observed complications, indicating the beneficial effect of the combination of growth hormone and platelet-rich plasma for individuals with knee osteoarthritis [8]. Kim et al. also conducted a study on a rabbit model of collagenase-induced osteoarthritis and found that intra-articular injection of growth hormone, in combination with hyaluronic acid, induced morphoangiogenesis and led to the formation of capillaries with unique characteristics, potentially contributing to joint repair and regeneration [22]. These findings suggest that synthetic human growth hormone may have additive effects when combined with other intra-articular treatments for osteoarthritis.

There were some causes on why does the cartilage regeneration do not happen. One possible cause could be inadvertent intravascular injection of hyaluronic acid, leading to rare but significant complications such as cutaneous necrosis. Repeated use of intra-articular injections, such as corticosteroids, may result in accelerated cartilage loss, potentially impeding the regenerative process. Moreover, the concentration and molecular weight of hyaluronan in synovial fluid are reduced in osteoarthritis, affecting joint lubrication and potentially impacting cartilage regeneration. Another factor to consider is the age of the patient, as older individuals may respond differently to treatments like platelet-rich plasma or hyaluronic acid injections, which could influence the regenerative outcomes [23].

Furthermore, the choice of substances injected, such as hypertonic dextrose, morrhuate sodium, or platelet-rich plasma, can stimulate growth factor and cytokine production, potentially influencing the regenerative capacity of the cartilage. Additionally, the accuracy of intra-articular injections is crucial to ensure that medications are delivered directly into the joint space, maximizing therapeutic benefits and minimizing complications that could impede cartilage regeneration [24,25].

MSCs administration for knee OA shows promising outcomes in improving knee joint function and reducing symptoms. We had conducted a previous study on MSCs administration on varying degree of knee OA, showing better improvement of IKDC and WOMAC without any improvement of T2 MRI mapping [26]. Its effectiveness and safety for knee OA treatment have been explored and summarized in a systematic review and meta-analysis, indicating that MSCs have the potential to be beneficial in managing knee OA [27]. Additionally, a study by conducted a Phase IIb, randomized,

 doi:10.20944/preprints202408.1003.v1

placebo-controlled clinical trial on the intra-articular injection of autologous adipose tissue-derived MSCs for knee OA, reporting positive results in improving knee joint function [9].

This is a novel study on the administration of combined compounds that are deemed effective and safe from the literature. When the three compounds were combined, it was expected to show improved results in pain level, functional scores, and cartilage thickness observed from the T2 mapping. However, only the VAS and WOMAC score showed a declining result during 12 months. These results aligned with previous study on MSC administration onto varying knee OA degree [26], however the result of combined agents onto T2 mapping remained insignificant. This warrants further studies regarding other treatment alternatives that can help the regeneration of articular cartilage of the knee.

5. Conclusions

Our findings demonstrate that while treatments such as hyaluronic acid, mesenchymal stem cells, and growth hormones individually show promise in clinical settings, their combined effects do not significantly enhance cartilage regeneration as measured by T2 MRI mapping. The clinical outcomes, primarily assessed through VAS and WOMAC scores, indicated a trend towards symptom relief across all treatment groups. Future research should focus on optimizing these therapies, possibly through novel combinations or enhanced delivery mechanisms, to improve their efficacy in cartilage regeneration and overall joint health. This study underscores the complexity of treating knee osteoarthritis and highlights the need for continued innovation in non-surgical interventions.

Author Contributions: Conceptualization, I.H.D.; methodology, I.H.D and J.P.; software, A.F., B.R.H. and I.G.H; validation, I.H.D and A.F.; formal analysis, I.H.D and J.P.; investigation, A.F., B.R.H. and I.G.H.; resources, I.H.D.; data curation A.F., B.R.H., and I.G.H.; writing—original draft preparation, I.H.D. and A.F.; writing—review and editing, B.R.H. and I.G.H; visualization, A.F., B.R.H., and I.G.H.; supervision, I.H.D.; project administration, I.H.D.; funding acquisition, I.H.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The National Research Priority Grant from Ministry of Research, Technology and Higher Education Indonesia.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the local Research and Ethics Committee (KET-1149/UN2.FI/ETIK/2019) of the Cipto Mangunkusumo Hospital, University of Indonesia.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available from the corresponding author upon request.

Conflicts of Interest:: The authors declare no conflicts of interest.

References

1. Hrnack SA, Barber FA. Managing the Pain of Knee Osteoarthritis. Phys Sportsmed. 2014;
2. Hatfield GL, Cochrane CK, Takacs J, Krowchuk NM, Chang R, Hinman RS, et al. Knee and Ankle Biomechanics With Lateral Wedges With and Without a Custom Arch Support in Those With Medial Knee Osteoarthritis and Flat Feet. J Orthop Res. 2016;
3. Perez-Huerta BD, Díaz-Pulido B, Pecos-Martín D, Beckwée D, Lluch-Girbés E, Fernández-Matías R, et al. Effectiveness of a Program Combining Strengthening, Stretching, and Aerobic Training Exercises in a Standing Versus a Sitting Position in Overweight Subjects With Knee Osteoarthritis: A Randomized Controlled Trial. J Clin Med. 2020;
4. Wang X, Huang L, Liu Y, Li J, Wu X, Li H, et al. Effects of Tai Chi Program on Neuromuscular Function for Patients With Knee Osteoarthritis: Study Protocol for a Randomized Controlled Trial. Trials. 2013;
5. Anandacoomarasamy A, March L. Current Evidence for Osteoarthritis Treatments. Ther Adv Musculoskelet Dis. 2010;
6. Tirmizi SH, Fakhr A, Amer A, Yusuf R, Sajjad K, Khan S. Comparison of Effectiveness of Intraarticular Injections of Hyaluronic Acid and Corticosteroids in the Treatment of Patients With Knee Osteoarthritis Symptoms. Pakistan Armed Forces Med J. 2021;

7. Palmieri B, Rottigni V, Iannitti T. Preliminary study of highly cross-linked hyaluronic acid-based combination therapy for management of knee osteoarthritis-related pain. Drug Des Devel Ther [Internet]. 2013 Jan 7 [cited 2024 Jul 7];7:7–12. Available from: https://www.dovepress.com/preliminary-study-of-highly-cross-linked-hyaluronic-acid-based-combina-peer-reviewed-fulltext-article-DDDT
8. Rahimzadeh P, Imani F, Faiz SHR, Alebouyeh MR, Azad-Ehyaei D, Bahari L, et al. Adding Intra-Articular Growth Hormone to Platelet Rich Plasma Under Ultrasound Guidance in Knee Osteoarthritis: A Comparative Double-Blind Clinical Trial. Anesthesiol Pain Med. 2016;
9. Lee WS, Kim K, Kim JS, Jin W. Intra-Articular Injection of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis: A Phase IIb, Randomized, Placebo-Controlled Clinical Trial. Stem Cells Transl Med. 2019;
10. Kenihan L, McTier L, Phillips NM. Patients' Expectations and Experiences of Stem Cell Therapy for the Treatment of Knee Osteoarthritis. Heal Expect. 2020;
11. Li JJ, Hosseini-Beheshti E, Grau GE, Zreiqat H, Little CB. Stem Cell-Derived Extracellular Vesicles for Treating Joint Injury and Osteoarthritis. Nanomaterials. 2019;
12. Kim SH, Djaja YP, Park YB, Park JG, Ko YB, Ha C. Intra-Articular Injection of Culture-Expanded Mesenchymal Stem Cells Without Adjuvant Surgery in Knee Osteoarthritis: A Systematic Review and Meta-Analysis. Am J Sports Med. 2019;
13. Bagge J, Hölmich P, Hammer FA, Nehlin JO, Vomstein K, Blønd L, et al. Successful Isolation of Viable Stem Cells From Cryopreserved Microfragmented Human Adipose Tissue From Patients With Knee Osteoarthritis – A Comparative Study of Isolation by Tissue Explant Culture and Enzymatic Digestion. J Exp Orthop. 2023;
14. Murphy M, Fink DJ, Hunziker EB, Barry F. Stem Cell Therapy in a Caprine Model of Osteoarthritis. Arthritis Rheum. 2003;
15. Dilogo IH, Primaputra MRA, Pawitan JA, Liem IK. Modified Masquelet technique using allogeneic umbilical cord-derived mesenchymal stem cells for infected non-union femoral shaft fracture with a 12 cm bone defect: A case report. Int J Surg Case Rep. 2017;34:11–6.
16. Emadedin M, Aghdami N, Taghiyar L, Fazeli R, Moghadasali R, Jahangir S, et al. Intra-articular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis. Arch Iran Med [Internet]. 2012 Jul;15(7):422–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22724879
17. Hamandi SJ, Al-Hussainy SD, Sabeeh SM. Gait Analysis Before and After Total Knee Replacement. Eng Technol J. 2019;
18. Xiao Q, Liu B, Zhao B. Gap Balancing Improve Squat Function and Knee Function: A Randomized Controlled Trial Comparing Gap Balancing and Measured Resection. J Orthop Surg Res. 2021;
19. Strauss EJ, Hart J, Miller MD, Altman RD, Rosen J. Hyaluronic Acid Viscosupplementation and Osteoarthritis. Am J Sports Med. 2009;
20. Buendía-López D, Medina-Quirós M, Marín MÁF. Clinical and Radiographic Comparison of a Single LP-PRP Injection, a Single Hyaluronic Acid Injection and Daily NSAID Administration With a 52-Week Follow-Up: A Randomized Controlled Trial. J Orthop Traumatol. 2018;
21. Polacco A, Zobel BB, Polacco M, Scarlata S, Gasparro F, Vescovo RD, et al. The Effect of Intra-Articular Hyaluronic Acid (Sinovial® One) on Knee Osteoarthritis: A Preliminary Study. Eur J Inflamm. 2013;
22. Kim SB, Kwon DR, Kwak H, Shin YB, Han HJ, Lee JH, et al. Additive Effects of Intra-Articular Injection of Growth Hormone and Hyaluronic Acid in Rabbit Model of Collagenase-Induced Osteoarthritis. J Korean Med Sci. 2010;
23. Pamuk Ç. Comparison of Intra-articular Hyaluronic Acid and Platelet-Rich Plasma Injection in Knee Osteoarthritis: Do the Results Differ in Geriatric Patients? A Retrospective Observational Study. Ann Geriatr Med Res [Internet]. 2022 Dec 31;26(4):340–6. Available from: http://www.e-agmr.org/journal/view.php?doi=10.4235/agmr.22.0090
24. Zhao AT, Caballero CJ, Nguyen LT, Vienne HC, Lee C, Kaye AD. A Comprehensive Update of Prolotherapy in the Management of Osteoarthritis of the Knee. Orthop Rev (Pavia) [Internet]. 2022 May 31;14(3). Available from: https://orthopedicreviews.openmedicalpublishing.org/article/33921-a-comprehensive-update-of-prolotherapy-in-the-management-of-osteoarthritis-of-the-knee
25. Chernchujit B, Tharakulphan S, Apivatgaroon A, Prasetia R. Accuracy comparisons of intra-articular knee injection between the new modified anterolateral Approach and superolateral approach in patients with symptomatic knee osteoarthritis without effusion. Asia-Pacific J Sport Med Arthrosc Rehabil Technol [Internet]. 2019 Jul;17:1–4. Available from: https://linkinghub.elsevier.com/retrieve/pii/S221468731830181X

 doi:10.20944/preprints202408.1003.v1

26. Dilogo IH, Canintika AF, Hanitya AL, Pawitan JA, Liem IK, Pandelaki J. Umbilical cord-derived mesenchymal stem cells for treating osteoarthritis of the knee: a single-arm, open-label study. Eur J Orthop Surg Traumatol [Internet]. 2020;30(5):799–807. Available from: https://doi.org/10.1007/s00590-020-02630-5
27. Song Y, Zhang J, Xu H, Lin Z, Chang H, Liu W, et al. Mesenchymal Stem Cells in Knee Osteoarthritis Treatment: A Systematic Review and Meta-Analysis. J Orthop Transl. 2020;