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Research Article

JOURNAL OF ORTHOPAEDICS AND SPORTS MEDICINE

ISSN: 2688-5115

Amniotic Derived Exosomes with Platelet Rich Fibrin Combined in the Treatment of Hip and Knee Osteoarthritis: A Retrospective 1-Year Follow-Up Study

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Abstract

Introduction: Osteoarthritis (OA) is a chronic degenerative joint disease characterized by the progressive breakdown of articular cartilage, subchondral bone changes, and synovial inflammation. Recent advances in regenerative medicine have focused on exosome-based therapies as a promising approach to address this challenge. Exosomes, small extracellular vesicles derived from various cell types, play crucial roles in intercellular communication, modulate inflammatory responses, promote chondrocyte proliferation, and enhance extracellular matrix synthesis. Exosomes derived from amniotic fluid have shown promise in regenerative medicine and tissue repair in preclinical studies and early case reports.

Methods: This is a retrospective database cohort study. The clinic database was searched for patients that had undergone exosome treatment combined with PRF for knee and hip OA between August 2023 and February 2024. 114 patients were included in the study. The data analyzed were 1-year follow-up WOMAC scores and sonographic pictures of the femoral joint cartilage in knees before- and 6 months after the treatment.

Results: Statistically significant positive changes in WOMAC scores (p = 0,0001) were presented. Significant sonographic changes to femoral joint cartilage were presented; a mean increase of 1,21 mm (p = 0,0001).

Conclusion: Amniotic derived exosomes combined with PRF is an effective treatment for hip and knee osteoarthritis. Significantly lowered WOMAC scores 1 year after the treatment, combined with a significant positive sonographic change in the femoral cartilage were presented.

Clinical relevance: This is the first human study using exosomes for the treatment of knee and hip OA with results demonstrating positive sonographic structural changes and improved clinical parameters.

Keywords: PRF; ALB-PRF; Exosomes, Osteoarthritis; Sonography; Regenerative medicine

Abbreviations: OA: Osteoarthritis; SVF: Stromal Vascular Fraction; PRF: Platelet Rich Fibrin; WOMAC: Western Ontario and McMaster Universities Arthritis Index; NSAIDs: nonsteroidal anti-inflammatory drugs; MSC: mesenchymal stem cells; TGF-β: transforming growth factor-beta; PDGF: platelet-derived growth factor; PPP: platelet-poor plasma; ALB-PRF: heat coagulated albumin-PRF; C-PRF: Concentrated PRF; SD: Standard deviation; ROM: Range of motion

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Citation: Torbjörn Ogéus. Amniotic Derived Exosomes with Platelet Rich Fibrin Combined in the Treatment of Hip and Knee Osteoarthritis: A Retrospective 1-Year Follow-Up Study. Journal of Orthopedics and Sports Medicine. 7 (2025): 129-137.

Received: February 18, 2025
Accepted: March 10, 2025
Published: March 18, 2025

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DOI:10.26502/josm.511500188

Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by progressive cartilage deterioration, subchondral bone remodeling, synovial inflammation, and pain, ultimately leading to functional impairment and disability [1]. It is the most prevalent form of arthritis, affecting over 500 million people worldwide, with a rising incidence due to aging populations and increasing obesity rates [2]. Despite extensive research into its pathophysiology, OA remains a significant clinical challenge with limited effective treatments. Current therapeutic approaches focus primarily on symptom relief rather than disease modification. Conservative management includes nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroid injections, physical therapy, and lifestyle modifications. However, these treatments do not halt disease progression, and surgical intervention, such as total joint arthroplasty, remains the definitive solution for end-stage OA [3]. Consequently, there is a growing interest in regenerative medicine approaches, including biologics such as platelet-rich fibrin (PRF) and amniotic-derived exosomes, to address OA at a molecular level.

Regenerative medicine aims to repair, regenerate, and restore damaged tissues using biological therapies that harness the body's intrinsic healing mechanisms. Over the past two decades, regenerative therapies have gained traction as potential disease-modifying treatments for OA. Among these, platelet-rich products, such as platelet-rich plasma (PRP) and PRF, have shown promise due to their ability to enhance cellular repair, modulate inflammation, and promote tissue regeneration [4]. PRF in particular, is a second-generation platelet concentrate that differs from PRP in its fibrin matrix composition and sustained release of growth factors, making it a more favorable option for long-term tissue healing [5]. Meanwhile, exosome-based therapy is an emerging field within regenerative medicine, offering a cell-free alternative to mesenchymal stem cell (MSC) therapy. Exosomes, extracellular vesicles derived from various cell types, play a crucial role in intercellular communication and tissue repair by delivering bioactive molecules such as proteins, lipids, and microRNAs [6].

Amniotic-derived exosomes have garnered attention for their potent regenerative properties. Amniotic fluid and amniotic membrane-derived cells contain a rich reservoir of bioactive molecules that contribute to tissue repair and immune modulation. Exosomes derived from these sources have demonstrated anti-inflammatory, anti-fibrotic, and chondroprotective effects in preclinical studies [7]. Unlike traditional cell-based therapies, exosomes do not pose risks related to cell viability, immune rejection, or tumorigenicity, making them a promising candidate for OA treatment [8]. Studies have suggested that amniotic-derived exosomes may exert their therapeutic effects by reducing synovial inflammation, preventing chondrocyte apoptosis, and promoting extracellular matrix synthesis [9]. These effects are primarily mediated by key microRNAs and growth factors involved in joint homeostasis, such as transforming growth factor-beta (TGF-β), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) [10].

PRF is an autologous blood-derived biomaterial that is increasingly recognized for its role in tissue regeneration. It is prepared by centrifuging whole blood without anticoagulants, resulting in a fibrin clot rich in platelets, leukocytes, and growth factors [11]. Unlike PRP, which requires activation and has a short duration of action, PRF has a sustained release of bioactive molecules, making it a superior option for prolonged tissue healing [12]. PRF has been shown to stimulate chondrocyte proliferation, reduce synovial inflammation, and enhance extracellular matrix synthesis, contributing to cartilage repair [13]. Additionally, its fibrin network provides a scaffold that supports cellular migration and retention, further enhancing tissue regeneration [14].

While both amniotic-derived exosomes and PRF independently show promise in OA treatment, their combination may offer synergistic effects that enhance therapeutic outcomes. The regenerative potential of exosomes lies in their ability to modulate cellular responses and deliver reparative molecules, while PRF provides a biocompatible scaffold that sustains the release of growth factors and supports cell proliferation. Together, these therapies may offer a comprehensive approach to cartilage repair by reducing inflammation, promoting matrix synthesis, and enhancing chondrocyte viability [15]. Recent studies have explored the combinatory use of exosomes and PRF in musculoskeletal conditions, demonstrating improved healing outcomes compared to either therapy alone [16].

Despite the growing interest in biologic therapies for OA, clinical data on the efficacy of amniotic-derived exosomes remain limited. While preclinical studies and small-scale clinical trials have reported promising results, larger retrospective analyses are needed to assess real-world outcomes, safety profiles, and long-term benefits. Retrospective studies provide valuable insights into patient-reported outcomes, radiographic improvements, and biomarkers associated with treatment response [17]. Furthermore, they allow for comparative analysis between different biologic treatments and standard care, facilitating the development of optimized therapeutic protocols for OA management.

As OA continues to impose a significant burden on global healthcare systems, there is an urgent need for innovative therapies that go beyond symptom relief and target the underlying disease mechanisms. Amniotic-derived exosomes and PRF represent promising regenerative approaches with

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DOI:10.26502/josm.511500188

the potential to modulate inflammation, enhance tissue repair, and slow OA progression. This retrospective study aims to evaluate the clinical efficacy and safety of these biologics in OA patients, providing critical data to support their integration into mainstream clinical practice. By leveraging the strengths of both exosome therapy and PRF, this study may pave the way for novel regenerative strategies that improve joint health and patient outcomes.

Materials and Method

Different types of autologous platelet concentrations and injection types have been investigated in the use against osteoarthritis and chondral defects. Prior publications from our clinic have indicated a positive effect on pain, stiffness and function symptoms. Especially where a combination of different techniques was utilized [18,19]. A standardized way of extending the effect of the platelets after injection is to heat a liquid platelet-poor plasma (PPP) layer, which extends the resorption properties of heated albumin (albumin gel) from 2 weeks to more than 4 months (ALB-PRF) [20].

Exosomes derived from stem cells can inherit some of the potential of their parent cells and have been proposed as a substitute for stem cell therapy in order to achieve a cell-free therapy option [21][22]. Different routes of administration have been utilized and the most common route in preclinical studies has been the intravenous (IV) injection [23].

Preparation of PRF, ALB-PRF and exosomes

40ml whole blood was collected from all patients before each of the PRF injections. Four 10ml Plastic, round-bottomed vacuum tubes were used to collect blood, after the collection, the tubes were spun on a horizontal swing-out bucket rotors centrifuge system. Two PRF protocols were used in the treatment series, including a Concentrated-PRF (C-PRF) protocol of 2000xg for 8 min and a Heat-Coagulated Albumin Gel -PRF (ALB-PRF) protocol of 2000x g for 8 min followed by heating and cooling down process before the intra-articular injection. The PRF and ALB-PRF protocols were utilized following standardized guidelines for PRF preparation published by Miron et al in 2019 [24].

The PRF injections performed were concentrated Platelet Rich Fibrin (C-PRF) [25] injections of 4ml, centrifuged at 2000×g for 8 min. While the ALB-PRF injections were 5ml ALB-PRF, spun at 2000xg for 8 min on a horizontal centrifuge, the albumin layer was heated according to the ALB-PRF protocol; 75 degrees for 10 min [26]. Before injection, the heat- coagulated albumin gel was cooled down to room temperature and mixed with the remaining C-PRF to create ALB-PRF. The centrifuge utilized in all PRF treatments was the Bio-PRF horizontal centrifuge (Bio-PRF, USA).

The Exosomes used were 4 trillion amniotic derived exosomes in a 1,5ml extracellular matrix (The center for Regenerative Medicine Laboratories, Miami, USA). Half of the matrix was diluted with 5 ml Saline for intravenous injection, while the other half was combined with PRF for intra-articular injection.

Administration of PRF and ALB-PRF

The patients underwent a series of intra-articular C-PRF and ALB-PRF injections in the osteoarthritic hip or knee. The first injection was combined with exosomes as described above, after one week one more intra-articular injection of the ALB-PRF type was performed and the treatment series was concluded with one more ALB-PRF injection one month after the second injection. All intra-articular joint injections were performed with ultrasound guidance to ensure needle placement inside the joint capsule.

Administration of the exosomes

Before administration, the exosomes were divided in half, where half of the injection (2 trillion exosomes) was injected intra-articular in the osteoarthritic joint combined with a C-PRF injection and the other half of the exosomes (the remaining 2 trillion) were diluted in 5 ml saline for a systemic injection intravenously (IV) using the IV-Push technique [27]. No allergic or adverse reactions were observed after any of the injections.

Instruments

The Western Ontario and McMaster Universities Arthritis Index (WOMAC) instrument evaluates three dimensions (pain, stiffness, and physical function). The instrument uses 24 items: pain (5), stiffness (2) and physical function (17) items. It produces three subscale scores, one for each dimension, and a total index score [28]. The WOMAC version used in this study uses 5-point Likert scale of 0–4, with lower scores indicating lower levels of symptoms or physical function. Each subscale is summarized to a maximum score; 20, 8, and 68 score points, for pain, stiffness and physical function respectively. WOMAC total index score or global score is calculated by summarizing the scores for the 3 dimensions [29]. The questionnaire is self-administered and takes approximately 5 min to complete.

Sonographic database

Ultrasound imaging has been demonstrated as an effective method for assessing the cartilage within the trochlear groove of the knee [30,31]. During the treatment protocol, sonographic (ultrasound) pictures of the affected knees were taken before the treatment, 3 months after and 6 months after the treatment. The cartilage in the trochlear groove were measured digitally in millimeter (mm) and compared on the before and after pictures and later added to the data file for each corresponding patient for easy database access. The hip joint's mechanical structure makes cartilage measuring

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DOI:10.26502/josm.511500188

difficult with ultrasound, therefore, only the sonographic data from the knee patients were included in the study.

Study design

This is a retrospective database cohort study. The clinic database was searched for patients that had undergone exosome treatment combined with PRF for Knee and Hip OA between August 2023 and February 2024.

According to the Ethics Commission of Stockholm, Sweden, retrospective database-based studies do not require ethical approval and patient informed consent whenever the data were acquired, saved and treated anonymously. This applies to the present study.

Subjects under the text describing the subjects

117 patients (56 female and 61 male) underwent amniotic derived exosome and PRF treatment for hip or knee OA at the clinic during the above-referred period.

On the first day of treatment, patients were informed about the data collection that routinely takes place in the clinic and were asked to give written consent for data collection. With patient's consent, all patients were given Western Ontario and McMaster Universities Arthritis Index (WOMAC) form to fill in, before the treatment started, after 6 months and after 1 year. Sonographic pictures were taken on all treated knee joints before the treatment, after 3 months and after 6 months.

The patients were included if they were scheduled for exosome and PRF treatment of knee and hip OA and if WOMAC data was available. The patients were excluded if all WOMAC data was not available or if they failed to come to the follow-up meetings or if they had another treatment before all WOMAC data was collected.

The inclusion criteria were met by 114 patients (55 female and 59 male) that were treated for hip or knee OA with exosomes and PRF. The data contains 59 knee OA patients and 55 hip OA patients.

Statistical analysis

Mean and standard deviation (SD) or frequencies (percentage) were used to characterize the sample. Normal distribution of the data was tested with the use of T-test and ANOVA tests. Demographic data comparisons between the groups (Hip vs. Knee OA) were performed based on data gathered before the treatment series with the use of t-tests for the independent samples.

To investigate whether there were significant differences in the mean WOMAC scores of patient's, multiple comparisons between the pairs of means were performed with t-tests and ANOVA tests for independent samples.

All statistical tests were performed with Prism 10 for Windows (Microsoft, USA). For all statistical tests, the 0.05 level of probability was set as the criterion for statistical significance.

Results

WOMAC data

The data of the 114 patients that met the inclusion criteria were analyzed. The patients in the sample were on average 51.42 ± 2.43 years old. There were no significant differences between the patients in the Hip- and Knee OA groups concerning their mean age (p = 0.2). The distribution of male and female patients was not significantly different between the knee OA and the hip OA groups (p = 0.9) (the demographic data be seen in Figure 1).

Table 1

Demographic data of the patients in the sample.

	Knee OA	Hip OA	p-value
n	59	55	
Age (years)	50,7 ± 2,27	52,15 ± 2,59	p = 0.23
Gender (%) Male	31 (52,5)	28 (50,9)	p = 0.90
Female	28 (47,4)	27 (49,0)	

Figure 1: Demographic data, Values are mean ± SD for age.

The treated knee patients (n=59) were analyzed using an ANOVA test and showed a significant improvement in total WOMAC score (p = 0,0001), a mean change from 49,3 on the baseline measurement, 15.0 on the 6-month follow-up, and 8.8 on the 1-year follow-up was seen (82,1% decrease in total).

The hip patients (n=55) were also analyzed using an ANOVA test and showed a similar positive change in WOMAC score (p = 0,0001) but with a higher baseline value where the mean changed from 53.7 to 15.5 after 6 months and 9.3 on the 1-year follow-up (82.7% decrease in total) (changes can be seen in Figure 2).

The pain dimension of the WOMAC scores was also analyzed using an ANOVA test. The pain levels in the treated knees were significantly lower (p = 0,0001) both on the 6-month follow-up and even lower on the 12-month follow-up with a total decrease of 88,7% (10.7 to 1.2) the largest decrease took place in the first 6 months after the treatment. The treated hips showed a similar change in pain levels after the treatment, but with a higher mean of pain level still after 12 months, the changes were statistically significant (p = 0,0001) and the change between the 6-month follow-up and the 12-month follow-up was smaller. The mean changed from 13.6 to 3.4 after 6 months and 2.8 after a year (79.4% decrease) (changes can be seen in Figure 3).

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DOI:10.26502/josm.511500188

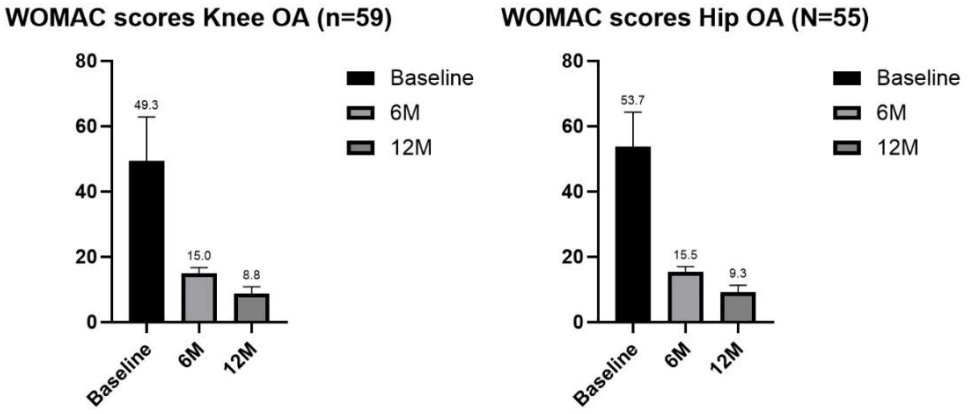

Figure 2: Mean values of WOMAC scores on the treated knees and hips with SD marked.

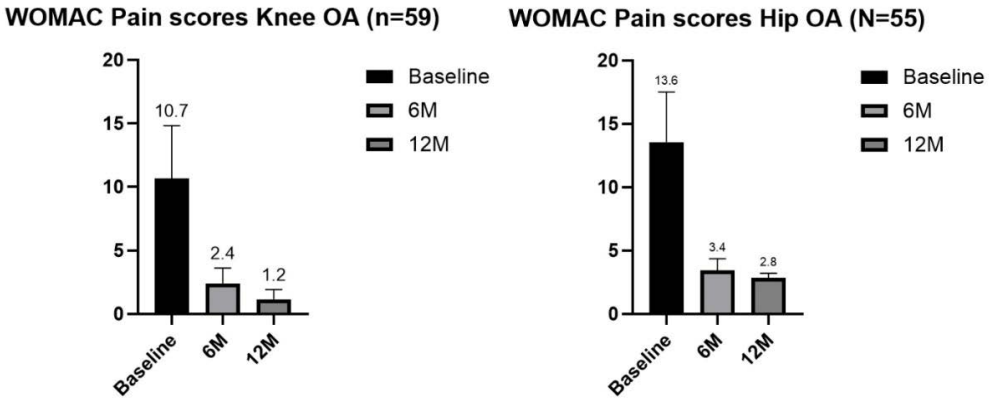

Figure 3: Mean values of WOMAC pain scores on the treated knees and hips with SD marked.

The stiffness dimension of the WOMAC scores was also analyzed using an ANOVA test. The stiffness levels in the treated knees were significantly lower on the 6-month follow-up and showed a 60% decrease on the 1-year follow-up (3.8 to 1.5, p = 0,0001). In the treated hips a similar significant reduction in stiffness was seen between the baseline and the 6-month follow-up (79%, p = 0,0001) (changes can be seen in Figure 4).

The last dimension of the WOMAC scores, physical function (PF) was also analyzed using an ANOVA test. The physical function scores in the treated knees were significantly improved on the 6-month follow-up and showed a total 82% improvement on the 12-month follow-up (34.6 to 6.1, p = 0,0001). The positive changes in physical function on the hips treated were also significant (85%, p = 0,0001) (changes can be seen in Fig5).

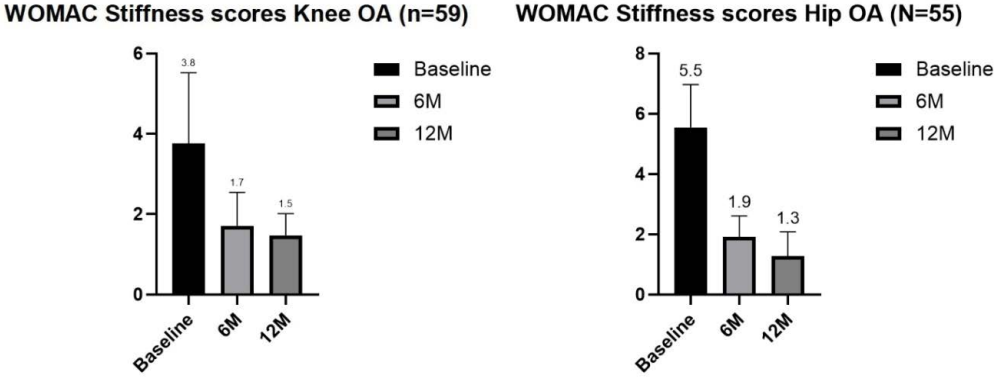

Figure 4: Mean values of WOMAC stiffness scores on the treated knees and hips with SD marked.

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DOI:10.26502/josm.511500188

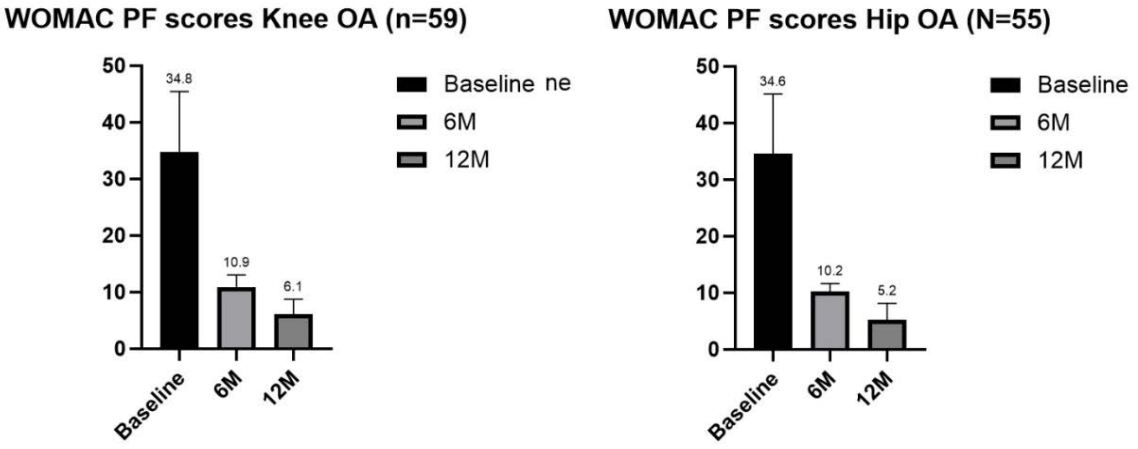

Figure 5: Mean values of WOMAC physical function scores on the treated knees and hips with SD marked.

There were no statistically significant differences between the genders in any of the analyzed dimensions.

Sonographic data

The sonographic data of the 59 knee patients that met the inclusion criteria were analyzed with a two-tailed unpaired T-test and compared, before- and 6 months after the treatment.

Due to the structural limitations the hip OA group could not be analyzed.

The data from the knees were analyzed and compared. The group showed a statistically significant increase in joint space, the knee patients showed a mean increase of 1.21mm ($p = 0{,}0001$) in the measured cartilage in the trochlear groove on the femur bone. changes can be seen in Figure 6).

Examples of the described sonographic changes with increased cartilage and repair in the trochlear groove can be seen in Figure 7.

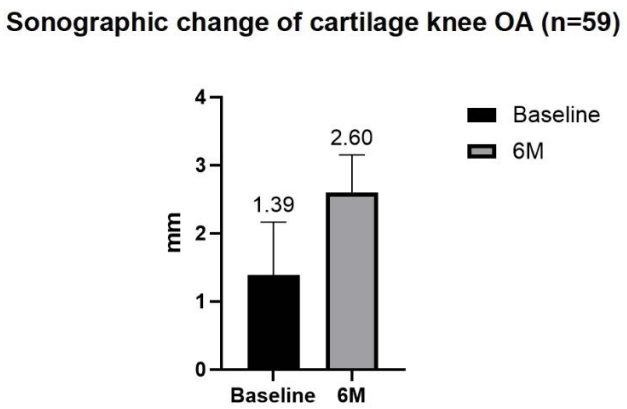

Figure 6: Mean values of cartilage increase in mm in the trochlear groove on the treated knees with SD marked.

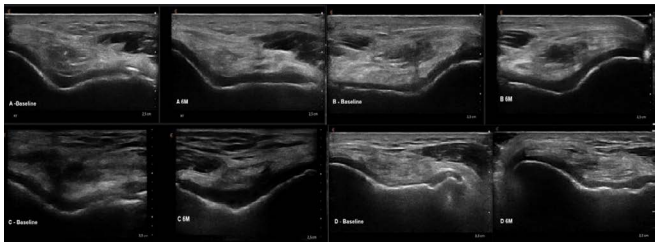

Figure 7: Sonographic pictures of treated OA knees, before and 6 Months after the Exosome and PRF treatment. A. Mean change from 2 mm trochlear cartilage to 3.4mm. B, Mean change from 1.5 mm trochlear cartilage to 2.8mm (a positive change can also be seen on the cartilages surface). C, Mean change from 2mm to 3,5mm and visible repair to the subchondral bone (damage to the subchondral bone can be seen on the baseline picture). D, Mean change from 1,2mm to a mean of 2.4mm.

Discussion

The data presented herein describe a successful novel use of a combination of amniotic-derived exosomes, PRF, and ALB-PRF for the treatment of osteoarthritis (OA). To our knowledge, this is the first study to evaluate long-term clinical outcomes using this amniotic derived exosomes and PRF. The results demonstrate significant improvements in patient-reported symptoms, including pain, stiffness, and physical function, as measured by the WOMAC index. Additionally, sonographic data from knee OA patients indicate a measurable increase in cartilage thickness, suggesting potential structural regeneration within the joint.

The observed reduction in WOMAC pain scores highlights the potential of regenerative medicine to offer sustained relief from OA-associated discomfort. The treated knee and hip OA groups both demonstrated statistically significant pain reduction over the 12-month follow-up period, with

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DOI:10.26502/josm.511500188

an 88.7% and 79.4% decrease, respectively. Notably, the majority of pain reduction occurred within the first six months post-treatment, aligning with prior studies suggesting that biologic therapies exert their most substantial effects within this timeframe [32]. The continued improvement at 12 months suggests a prolonged therapeutic effect, likely due to the sustained release of growth factors from the ALB-PRF and the biological activity of exosomes in modulating inflammation and cartilage repair [33].

Similarly, the stiffness and physical function domains of the WOMAC index showed marked improvements, with an 82% improvement in knee function and an 85% improvement in hip function at 12 months. These results are consistent with previous studies that have explored PRF-based therapies for OA, reinforcing the hypothesis that PRF provides an extended release of bioactive molecules that enhance tissue healing and joint mobility [34]. The significant improvement in physical function suggests that regenerative therapies may enhance joint biomechanics and overall mobility in OA patients, potentially delaying the need for invasive surgical interventions such as total joint replacement.

Sonographic data provided additional insights into the regenerative effects of the combination therapy. The increase in cartilage thickness in knee OA patients (mean increase of 1.21 mm) supports the hypothesis that PRF and exosomes may contribute to cartilage repair [35]. Given that OA is characterized by progressive cartilage degradation, the observed increase in joint space is an encouraging finding that warrants further investigation. It is noteworthy that similar measurements could not be obtained in hip OA patients due to anatomical constraints, highlighting the need for advanced imaging modalities such as MRI in future studies.

The use of both intra-articular and intravenous exosome administration may have contributed to the observed therapeutic effects. The intra-articular route ensures localized delivery to the affected joint, while systemic IV administration may exert broader immunomodulatory effects. This dual approach aligns with emerging evidence that suggests exosomes exert both local and systemic benefits in regenerative medicine applications [36]. However, further research is needed to optimize dosing protocols and evaluate the relative contribution of each administration route to clinical outcomes.

While this study provides promising data, several limitations should be acknowledged. First, the retrospective nature of the analysis introduces potential selection bias, as patients were not randomized or compared to a control group receiving standard OA treatment. Additionally, while WOMAC scores provide valuable patient-reported outcome data, they are inherently subjective and may be influenced by external factors such as patient expectations or concurrent lifestyle changes. The lack of MRI-based cartilage assessment also limits the ability to fully quantify structural changes within the joint. Future prospective, randomized controlled trials with larger sample sizes and advanced imaging modalities are needed to validate these findings and establish standardized protocols for biologic OA treatment.

In comparison with a former study on hip and knee OA treated with Stromal Vascular Fraction (SVF) combined with PRF published from our clinic last year [37], some interesting differences between the two treatments can be pointed out:

1. The exosome and PRF treatment show similar WOMAC score results, however the initial pain and stiffness reduction can be seen earlier than with the SVF and PRF treatment.
2. The Initially faster recovery and improvement slows down and the WOMAC result after one year is slightly inferior to the results experienced after SVF and PRF treatment.
3. There is a positive change to the cartilage in the trochlear groove in the treated knees, however, it is about half of the joint space increase seen after SVF and PRF treatment with radiographic imaging (1,21mm compared to 2.02mm).

In summary, the combined exosome and PRF treatment presented, offer a minimally invasive, low risk treatment to patients with OA in knee or hip joints as an alternative to total joint replacement surgery or stem cell treatments, without convalescence and at a much lower cost.

Despite the limitations mentioned, the findings of this study support the potential integration of exosome and PRF therapies into OA management strategies. The observed improvements in pain, function, and cartilage integrity suggest that these regenerative approaches may offer a viable alternative to conventional symptom-based treatments. Given the growing burden of OA worldwide, continued research into biologic therapies is essential to develop effective disease-modifying interventions that improve long-term patient outcomes.

In our clinic more than 300 treatments with amniotic derived exosome injections have been performed prior to the publication with promising results, no adverse effects or allergic reactions have been observed.

Conclusion

Amniotic derived exosomes combined with PRF is an effective treatment for knee and hip osteoarthritis. Significantly lowered WOMAC scores 1 year after the treatment, combined with a significant positive sonographic change in the femoral cartilage were presented.

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DOI:10.26502/josm.511500188

Declarations

Ethics Approval

According to the Ethics Commission of Stockholm, Sweden, retrospective database-based studies do not require ethical approval and patient informed consent whenever the data were acquired, saved and treated anonymously. This applies to the present study.

The study was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate: Not applicable.

Consent for Publication

This manuscript does not contain any individual person's data. All data exposed in this manuscript was anonymized.

Funding

The author received no financial support for the research, authorship, and/or publication of this article.

Acknowledgements

The author was the main and only contributor to the manuscript.

Competing Interests

The author declares that he has no competing interests.

Authors' contributions

All texts, design, literature review and drafting of this study were done by TO, responsible for the submitted manuscript.

Availability of data and materials

All data generated or analyzed during this study can be provided by the corresponding author upon reasonable request and is available for review by the Editor-in-Chief of this journal.

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Torbjörn Ogéus DC, PgD, MSc, ScA*., J Ortho Sports Med 2025
DOI:10.26502/josm.511500188

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Citation: Torbjörn Ogéus. Amniotic Derived Exosomes with Platelet Rich Fibrin Combined in the Treatment of Hip and Knee Osteoarthritis: A Retrospective 1-Year Follow-Up Study. Journal of Orthopedics and Sports Medicine. 7 (2025): 129-137.