




Article

Office-Based Intraosseous Infiltrations of PRGF as an Effective Treatment for Knee Osteoarthritis: A Retrospective Observational Clinical Study

Antonio Ríos Luna ^{1,*} , Homid Fahandezh-Saddi Díaz ², Manuel Villanueva Martínez ³, Roberto Iglesias ¹, Roberto Prado ⁴ , Sabino Padilla ⁴ and Eduardo Anitua ⁴ 

- ¹ Department of Traumatology and Orthopedic Surgery, Clínica Orthoindal, 04004 Almería, Spain; iglroberto@gmail.com
² Department of Orthopedic Surgery, Hospital Universitario Fundación Alcorcón, 28922 Alcorcón, Spain; homidfsd@hotmail.com
³ Department of Traumatology and Orthopedic Surgery, Avanfi Institute, 28015 Madrid, Spain; mvillanuevam@gmail.com
⁴ Regenerative Medicine Laboratory, BTI—Biotechnology Institute I MAS D, 01007 Vitoria, Spain; roberto.prado@bti-implant.es (R.P.); sabino.padilla1958@gmail.com (S.P.); eduardo@fundacioneduardoanitua.org (E.A.)
* Correspondence: antonioriosluna@gmail.com

Abstract: The aim of this study was to explore and assess office-based ultrasound-guided intraosseous and intra-articular infiltrations of plasma rich in growth factors (PRGF) in patients with moderate and severe knee osteoarthritis (KOA). Seventy-nine patients (30 women and 49 men) with grade 3–4 KOA according to the Kellgren–Lawrence classification participated in the study. All patients were treated with a minimally invasive technique using local anesthesia WALANT (wide-awake local anesthesia no tourniquet) in the ambulatory setting. A PRGF intra-articular infiltration and two intraosseous infiltrations in the tibial plateau and femoral condyle were performed weekly for a total of three sessions. The evaluation of the results was carried out using knee injury and osteoarthritis outcome score (KOOS) at baseline and post-treatment. After a follow-up period of 11 months (median) [interquartile range, 7–14], all the KOOS domains showed statistically significant improvement ($p < 0.001$). Moreover, 88% of the patients showed a pain reduction of at least 10 points (minimally clinically important improvement) from pre- to post-treatment. Our retrospective study using the in-office procedure of ultrasound-guided combination of intra-articular and intraosseous infiltrations of PRGF is a safe and efficacious approach for the treatment of grade 3–4 knee osteoarthritis.

Keywords: intraosseous; osteoarthritis; PRGF; platelet-rich plasma



Citation: Ríos Luna, A.; Fahandezh-Saddi Díaz, H.; Villanueva Martínez, M.; Iglesias, R.; Prado, R.; Padilla, S.; Anitua, E. Office-Based Intraosseous Infiltrations of PRGF as an Effective Treatment for Knee Osteoarthritis: A Retrospective Observational Clinical Study. *J. Clin. Med.* **2023**, *12*, 4512. <https://doi.org/10.3390/jcm12134512>

Academic Editor: Nicola Maffulli

Received: 15 June 2023

Revised: 28 June 2023

Accepted: 4 July 2023

Published: 6 July 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Knee osteoarthritis (KOA) is a painful, chronic low-grade inflammatory disease in which the whole synovial joint tissue homeostasis is dysregulated [1], with the cartilage and synovium suffering the consequences of subchondral bone (SCB) biochemical disruptions in a loop manner among whole synovial joint tissues [1–7]. KOA is a highly prevalent disease, and the latest data suggest that it affects 37% of people over 60 years of age [8]. As a disease affecting the whole synovial joint tissues, the symptomatic treatment of KOA should target as many tissues as possible that are involved in the generation of pain, stiffness, and swelling that lead to mechanical failure, thereby limiting daily activities and quality of life [6,9].

Recently, mechanism-oriented therapeutic products and delivery strategies have emerged in the field of regenerative pain medicine to treat KOA pain and disabilities, among which autologous blood-derived products [10] are delivered via a dual approach by combining intra-articular and intraosseous routes [11,12]. Among these autologous

biologics, plasma rich in growth factors (PRGF) is a type of platelet-rich plasma (PRP) with moderate enrichment in platelets and an absence of leukocytes and erythrocytes [13]. The immunomodulatory, anti-inflammatory, and antialgic effect of this bioresorbable and biocompatible versatile therapeutic tool is based on the gradual and progressive release of IGF-1, TGF- β , HGF, PDGF, and VEGF from the myriad of growth factors (GFs) contained in platelets and plasma. These biomolecules are trapped in the functionalized fibrin matrix after PRGF activation [14]. PRP, as well as isolated growth factors such as HGF, IGF-1, PDGF, and TGF- β which are contained in PRGF, have been reported to inhibit the NF κ B inflammatory pathway in chondrocytes, synoviocytes, and macrophages [15–19], a common therapeutic target to dampen the expression of catabolic, proinflammatory, and senescent genes involved in osteoarthritis [20,21].

In line with this, a growing number of clinical studies have demonstrated favourable pain relief and improvement in the quality of life using a combination of intra-articular and intraosseous infiltrations of platelet-rich plasma (PRP) for the treatment of moderate and severe KOA [12,22–25]. This approach has been shown to be safe, with long-term clinical and functional benefits, and the procedure is easy to implement in the ambulatory setting using ultrasound guidance assisted with wide-awake local anesthesia no tourniquet (WALANT) technique, without sedation [26].

The aim of this study was to explore and assess this office-based ultrasound-guided intraosseous and intra-articular infiltrations of plasma rich in growth factors (PRGF) treatment in patients with moderate and severe KOA using the baseline and post-treatment knee injury and osteoarthritis outcome score (KOOS) as a means of evaluating the therapy.

Thus, the hypothesis of our study was that intraosseous and intra-articular infiltrations of PRGF improve pain and quality of life in patients with KOA on a clinically relevant basis.

2. Materials and Methods

2.1. Study Design and Data Source

This retrospective study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines (Supplementary Table S1) [27]. The study protocol (AR-01-ER-22) was approved by the Institutional Review Board (CEIm-E) in accordance with the revised World Medical Association Declaration of Helsinki, amended in 2013 in Brazil [28]. In order to obtain the data for this study, a pseudonymized database of patients suffering from KOA treated with PRGF infiltrations in the Clínica Orthoindal between January 2020 and September 2022 was retrospectively reviewed.

The inclusion criteria for the study were as follows: (1) patients over 18 years old from both sexes; (2) patients diagnosed with grades 3–4 KOA according to the Kellgren–Lawrence classification; (3) patients treated with office-based intraosseous and intra-articular infiltrations of PRGF; (4) patients who completed the KOOS (knee injury and osteoarthritis outcome score) evaluation scale prior to PRGF intervention and at the end of the follow-up; and (5) a minimum follow-up of six months. The absence of complete data on the variables to be studied was the exclusion criterion.

2.2. PRGF Preparation

PRP was prepared according to PRGF-Endoret technology (BTI Biotechnology Institute, Vitoria, Spain). Briefly, peripheral venous blood was withdrawn into eight 9-mL collection tubes containing 0.4 mL of 3.8% (wt/vol) trisodium sodium citrate as an anticoagulant. Next, the blood was centrifuged at 580 g for 8 min at room temperature. The upper volume of the plasma was discarded. The 2 mL plasma fraction (F2), located just above the sediment red blood cells but not including the buffy coat, was collected. PRGF (F2) has a moderate enrichment of platelets and is free of leukocytes and erythrocytes, so it can be classified as pure PRP (P-PRP). Based on the aforementioned features of PRGF, and in accordance with the latest coding system [29], PRGF is a 24-00-11 PRP. In addition, Anitua et al. [13] recently published a comprehensive summary of the different classifications of PRGF.

To initiate clotting and platelet activation following the instruction of the manufacturer, calcium chloride (10% wt/vol) was added to liquid PRGF F2 just before each infiltration (20 μ L/mL PRGF).

2.3. PRGF Infiltration Procedure

The office-based intraosseous PRGF infiltration procedure was recently described by Rios et al. [26]. The entire treatment, including anesthesia and infiltration, was carried out on an outpatient basis (office-based procedures). Briefly, after blood extraction, the patient was positioned on the operating table in the supine position. Anesthesia was performed according to the WALANT technique and following the “hole-in-one” approach. A total of 10 mL of a mixture of 1% lidocaine (10 mg/mL) and epinephrine 1:100,000 (0.005 mg/mL) was prepared, followed by the addition of 1 mL of 8.4% sodium bicarbonate. This solution was infiltrated subcutaneously into the two entry points of the intraosseous infiltrations. The whole procedure was assisted with ultrasound guidance. The first infiltration was intra-articular, consisting of 8 mL of freshly activated PRGF and using the parapatellar external approach with a 21 G needle. Before proceeding with the intraosseous infiltrations, it was crucial to delay the injection of the local anesthesia by at least 30 min. Once the effectiveness of the anesthesia was assessed, drilling was performed with a 15 G trocar-biopsy needle system on the tibial entry. The trocar was placed 2 cm distal to the articular line with an inclination of 45° and 1.5 cm deep into the bone. Subsequently, 2 mL of the recently activated PRGF was gradually infiltrated via the trocar. The next step was the intraosseous infiltration of PRGF into the femoral condyle, with the trocar placed 2 cm proximal to the articular line with an inclination of 30° and advanced 1.5 cm deep into the subchondral femoral bone. Thus, 2 mL of the recently activated PRGF was infiltrated progressively via the trocar. The patient was able to easily get up from the operating table and begin walking immediately.

The complete treatment, namely, the intra-articular infiltration and the two intraosseous infiltrations in the tibial plateau and femoral condyle, was performed weekly for a total of three sessions.

2.4. Patient Evaluation and Follow-Up

The evaluation of the results was carried out using patient-reported outcomes. We used the validated KOOS (knee injury and osteoarthritis outcome score) scale, which was developed in the nineties and first published in 1998 by Ross et al. [30]. It is composed of five subscales that are measured separately: (1) KOOS pain (nine items); (2) KOOS symptoms with seven items, including other symptoms such as swelling, restriction of motion, and mechanical symptoms; (3) KOOS activities of daily living (17 items that measure the degree of involvement in activities of daily living); (4) KOOS sports/recreation (five items that reflect the degree of engagement at a more physically demanding level than daily life); and (5) KOOS quality of life (four items that measure the quality of life, social and mental aspects, such as lifestyle changes). A 5-point Likert scale was used for each item. Scores were converted to a 0–100 numeric scale, where 0 corresponded to extremely bad knee problems and 100 corresponded to perfectly healthy knees. Following the directions of Ross et al., an aggregate score was not calculated [30]. A reduction of at least 10 points on the KOOS pain scale, with respect to the pretreatment pain level, was considered a minimally clinically important improvement (MCII) [24]. Patients completed the KOOS questionnaire prior to treatment and at the end of follow-up, which, according to the inclusion criteria, was at least six months.

The safety of the procedure was also evaluated. All complications and adverse events were also gathered from the medical records of the patients.

2.5. Statistical Analysis

Descriptive data were presented as frequencies and percentages. The results of the questionnaires were presented as medians (interquartile range (IQR)). All data were tested

for normality using the Shapiro–Wilk test. The box-and-whisker plots were generated following the Tukey style: the boxes show the median and the IQR, while the whiskers indicate the 25th percentile- $1.5 \times$ the IQR and the 75th percentile- $1.5 \times$ IQR. The round points outside the whisker range indicate the outliers [31]. The Wilcoxon matched-pairs signed rank test was used to observe changes in the KOOS domains pre- and post-treatment. Differences were considered statistically significant at a value of $p < 0.05$. Statistical analyses were performed using GraphPad Prism, version 9 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Patient Characteristics

This study included 79 patients (30 women and 49 men), and 85 knees were treated with three weekly sessions of a combination of three intraosseous and intra-articular infiltrations of PRGF in the ambulatory setting assisted with the WALANT technique. The severity of KOA assessed by the Kellgren–Lawrence OA grade was grade 3 in 38 patients (44.7%) and grade 4 in 47 patients (55.3%). The median follow-up period was 11 months, with an interquartile range of 7–14 months. The baseline demographic characteristics of patients who participated in this study are shown in Table 1.

Table 1. Baseline demographic characteristics of patients.

Knees (<i>n</i>)	85
Patients (<i>n</i>)	79
Gender	
Female (<i>n</i> , %)	30 (38.0%)
Male (<i>n</i> , %)	49 (62.0%)
Age (years, mean \pm SD)	60.9 \pm 9.6
Height (cm, mean \pm SD)	170.3 \pm 9.0
Weight (kg, mean \pm SD)	80.0 \pm 17.6
Body mass index (kg/m ² , mean \pm SD)	27.5 \pm 5.2
Kellgren and Lawrence OA grade (<i>n</i> , %)	
Grade 3	38 (44.7%)
Grade 4	47 (55.3%)
Treatment side (<i>n</i> , %)	
Right (<i>n</i> , %)	55 (64.7%)
Left (<i>n</i> , %)	30 (35.3%)
Follow-up period (months, median (IQR *))	11 (7–14)

* IQR, interquartile range.

3.2. Clinical Outcomes

The assessment of patients after the follow-up period showed a statistically significant improvement in each of the domains of the KOOS questionnaire. Patients showed significant pain reduction ($p < 0.001$), symptoms ($p < 0.001$), activities of daily living ($p < 0.001$), sports/recreational activities ($p < 0.001$), and quality of life ($p < 0.001$) according to the KOOS score (Figure 1) (Table 2). The percentage of patients who showed pain reduction of at least 10 points (MCII) from pre- to post-treatment (6–24 months) was 88%, whereas 11.8% (10 knees) did not reach MCII improvement (Figure 2). It can be noted that the non-responders to treatment (10 knees) were evenly distributed throughout the follow-up period. Of the 10 knees that did not reach MCII, only 3 worsened, with a negative KOOS PAIN difference (Figure 2).

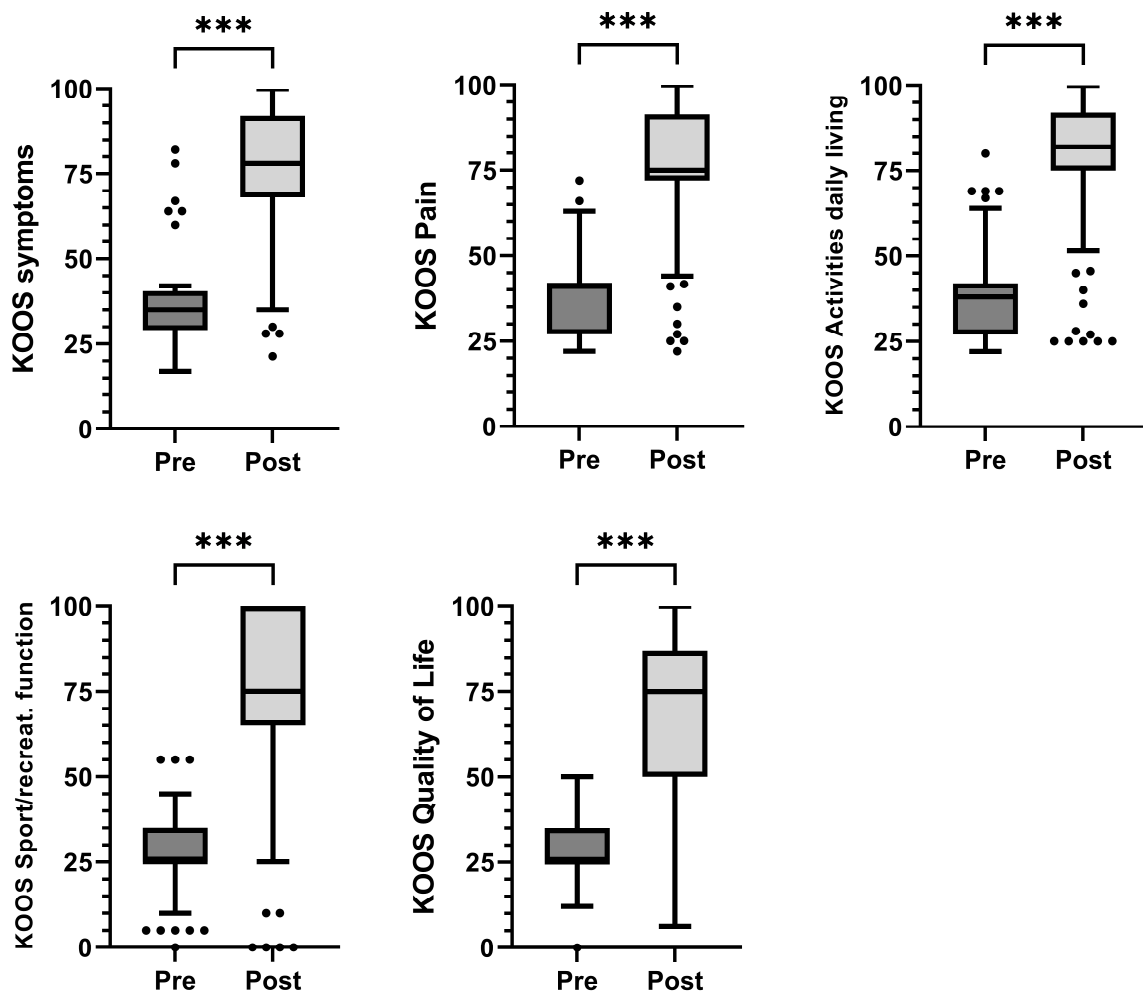


Figure 1. Results of the KOOS questionnaires. The results of the five subscales are shown graphically: KOOS symptoms, KOOS pain, KOOS activities of daily living, KOOS sports/recreation, and KOOS quality of life ($n = 85$ knees). *** indicates $p < 0.001$.

Table 2. Comparison of changes in KOOS.

	Pre-Treatment	Post-Treatment	<i>p</i> Value *
Domain			
Pain	41 (27–42)	75 (72–91.4)	<0.001
Symptoms	35 (29–40.5)	78 (68–92)	<0.001
Activities of Daily Living	38 (27–42)	82 (75–92)	<0.001
Sports/recreation	25 (25–35)	75 (65–100)	<0.001
Quality of life	25 (25–35)	75 (50–87)	<0.001

Values are expressed as median (interquartile ranges). * Wilcoxon matched-pairs signed rank test.

3.3. Adverse Events

Seven patients reported stiffness and pain during the first 24 h, two patients had knee swelling, and eight patients showed signs of inflammation at the entry points of the trocar. All these events were mended satisfactorily with oral pharmacological treatment with acetaminophen, which was allowed in the study.

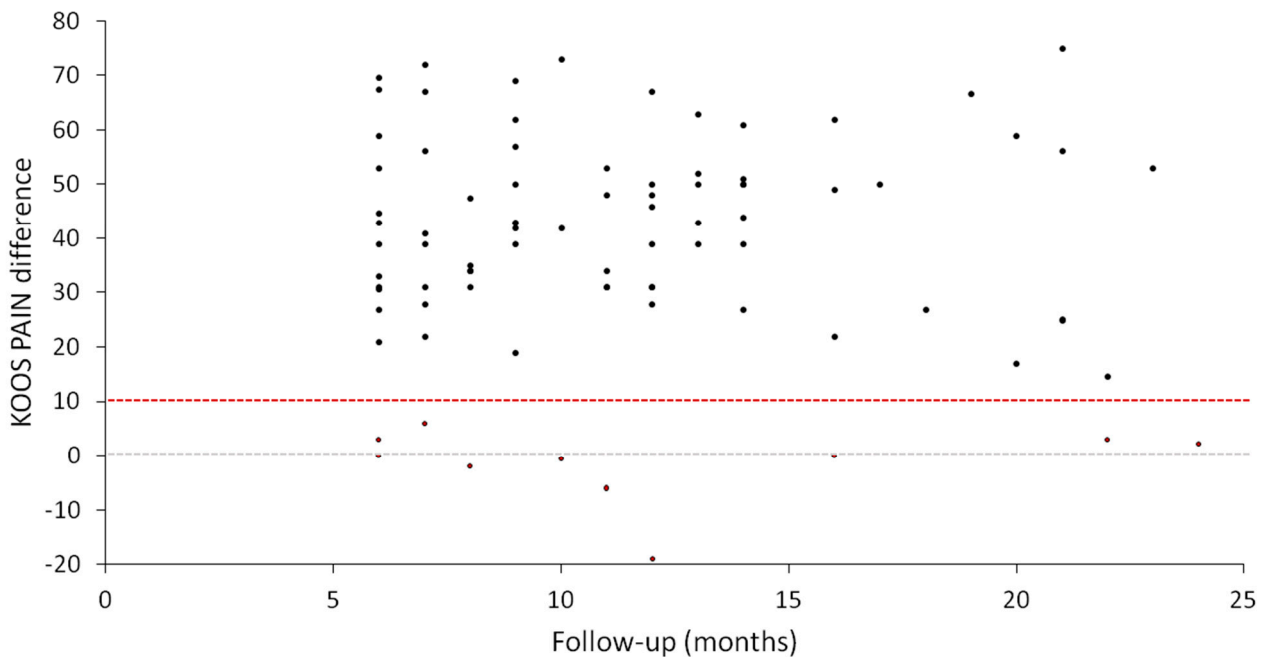


Figure 2. Graphical representation of the improvement in the KOOS pain scale versus follow-up time for the 85 knees included in the study. Seventy-five knees improved with treatment and exceeded the MCII for pain (black dots above the dotted red line), whereas only 10 knees (11.8%) were non-responders to treatment and did not reach this level of improvement (red dots). Each dot represents one knee in the study.

4. Discussion

This study shows for the first time the clinical feasibility, safety, and efficacy of office-based ultrasound-guided intraosseous and intra-articular infiltration of the PRGF procedure in patients with grade 3–4 KOA. Patients improved significantly in each of the domains covered by the KOOS questionnaire after a median of 11 months of follow-up after PRGF treatment. The results of this study are in agreement with several clinical studies using intra-articular [32–36] or a combination of intraosseous and intra-articular PRP infiltrations and reporting a clinical improvement in pain and functionality of patients assessed by KOOS subscales [12,22,24,25,37], even delaying the need for knee arthroplasty between 1.5 and 5 years [23]. Compared to other treatments, two recent systematic reviews of level 1 evidence studies concluded that PRP is more effective than hyaluronic acid and at least as effective as bone marrow aspirate concentrate in the treatment of KOA [38,39].

By using the intra-articular route with this growth factor delivery system, PRGF reaches the synovial membrane (SM) and articular cartilage, two tissues that are sometimes inefficiently targeted by systemic drug delivery. Moreover, the intra-articular route circumvents systemic toxicity and its side effects, presents an excellent bioavailability, and does not entail molecular size limitation, in contrast to the systemically delivered molecules entering the joint via the capillaries of the subsynovium [40,41]. PRGF operates as a dynamic liquid scaffold with a fibrin network from which GFs are gradually released into the tissue, thereby overcoming the challenge of short joint dwell time of drugs, as the lymphatic drainage clears proteins within a few hours [42,43]. However, the role of the SCB in the pathophysiology of OA [44–46] is now well recognized, which might make the intra-articular route insufficient to tackle all the joint tissues involved in KOA. This realization has led to a new strategy to safely deliver PRGF to the damaged synovial joint by combining intra-articular and intraosseous infiltrations as an *in situ* biological ‘joint centric’ approach to treat osteochondral defects, osteochondritis dissecans, osteonecrosis, bone marrow edema-like lesions, and OA [11,44]. This “joint-centric” approach to treating severe knee OA conveys a pool of growth factors, including TGF- β , HGF, SDF-1, and IGF-1,

naturally embedded in the cell-friendly scaffold of fibrin into four synovial joint tissues, namely, articular cartilage, synovial fluid, SM, and subchondral bone (SCB) [11,12,44]. The importance of treating KOA as a whole-organ disease has recently been demonstrated by the finding that the microchannel network of the subchondral bone is already modified and shows collective structural deterioration in the early stages of KOA [47]. Significantly, the cells and extracellular matrix of knee joint tissues are dysregulated, rendering SM and SCB a source of pain and inflammatory cytokines [7,10,48,49]. In this respect, the administration of PRP to the osteoarthritic knee and hip has been shown to exert analgesic and immunomodulatory effects [22,25,34,50–52], and it has been reported to modify the senescence phenotypes of mesenchymal stem cells (MSC) and osteogenic lineage at the subchondral bone level in mice and humans [53–55]. Importantly, the senescence of stromal cells in synovial joints has been associated with inflammation and pain [6,49]. In line with this, PRP might exert a senolytic activity on subchondral MSCs by restoring the osteogenic microenvironment and TGF- β homeostasis [56,57], thereby contributing to the attenuation or elimination of pain and even operating as a disease-modifying approach [49,58,59], but this remains to be confirmed by more histological and image-based evidence in humans. In addition, SDF-1, TGF- β , and fibronectin, which are part of the PRP biomolecules embedded in the fibrin matrix, exert chemotactic and chondrogenic effects and influence endogenous MSC homing from synovial fluid [50]. Accordingly, PRGF-like platelet-rich plasma containing a low platelet concentration and very few leukocytes has been shown to positively influence migration, proliferation, and chondrogenic differentiation of cultured human subchondral mesenchymal progenitor cells [60–62]. In line with this, IGF-I and -II, PDGF, SDF-1, TGF- β , CCL5, and fibronectin, all soluble morphogens trapped in the PRGF fibrin matrix, have been reported to influence recruitment and homing, and the chondrogenic differentiation of chondroprogenitor or MSCs from subchondral mesenchymal progenitor cells [61,63]. Finally, Sanchez et al. [44] suggested that the balanced ratio between platelet-secreted TGF- β 1 and VEGF, and plasma growth factors such as IGF-1 and HGF, all conveyed by PRP intraosseous infiltrations, might reduce or buffer the excess of TGF- β in SCB and restore HGF activity synthesized by SCB cells [44]. This modulatory effect of PRP on the TGF- β 1 signalling pathway might contribute to the shrinking of the fibrovascular tissue that replaces the bone marrow of OA subchondral bone. This fact might be explained by the antifibrotic mechanism already reported to be exerted by PRP on several cell phenotypes [64–66]. This new PRP-induced homeostatic balance between TGF- β 1 and HGF [67,68] reduces the synthesis of NGF, VEGF, and other inflammatory mediators, thereby resulting in the modulation of pathologic fibrovascular tissue and the reduction of pain and hyperalgesia [69].

Importantly, this in-office procedure of intraosseous infiltrations with PRGF represents a step forward in the cost-effectiveness of the treatment of knee osteoarthritis that healthcare systems and surgeons should take into account as a significant therapeutic tool to safely treat moderate and severe KOA [70–72].

However, this study presents several limitations. The first limitation is inherent in the study design, as this was a retrospective study based on the usual clinical practice. Another limitation was the mid-term follow-up of 11 months. In order to consider this treatment as a disease-modifying approach, the follow-up should have been longer and time-homogeneous for all the patients participating in the study. However, it was reported that bone marrow lesions disappeared after this treatment [24,36], and several *in vivo* studies reporting structural modifications after PRP administration reinforce this potential effect [55,59,73]. Another drawback is the absence of a control group, as it has been shown that intra-articular treatment entails a placebo effect [74]. A third limitation is the lack of X-ray or MRI studies, which would have been very useful to confirm eventual changes in the SCB after ultrasound-guided intraosseous and intra-articular infiltrations of PRGF.

5. Conclusions

In summary, considering the limitations of this retrospective study, this study shows that intra-articular and intraosseous PRGF infiltrations performed in an office setting are safe and effective in the treatment of moderate-to-severe KOA. Further randomized clinical trials are needed to confirm the data obtained in the present usual clinical practice study.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jcm12134512/s1>, Supplementary Table S1: Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement checklist.

Author Contributions: Conceptualization, A.R.L.; methodology, A.R.L., H.F.-S.D., M.V.M., R.I., R.P., E.A. and S.P.; writing—original draft, S.P. and R.P.; writing—review and editing, A.R.L., H.F.-S.D., M.V.M., R.I., R.P., E.A. and S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Institutional Review Board of CEIm-E (protocol code AR-01-ER-22).

Informed Consent Statement: Oral and written information about the medical treatment was provided to all patients. For this retrospective study, the Ethics Committee waived informed consent for data collection for patients who had completed follow-up, but required it for those who were still under follow-up (AR-01-ER-22).

Data Availability Statement: All the obtained data used to support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare that E.A. is the Scientific Director of, and S.P. and R.P. are scientists at the BTI—Biotechnology Institute I MAS D, a biomedical company that investigates the fields of regenerative medicine and PRGF-Endoret technology. The other authors state that they have no conflicts of interest.

Abbreviations

IQR	Interquartile range
KOA	Knee osteoarthritis
GFs	Growth factors
KOOS	Knee injury and osteoarthritis outcome score
MCI	Minimally clinically important improvement
MSC	Mesenchymal stem cells
PRGF	Plasma rich in growth factors
PRP	Platelet-rich plasma
SCB	Subchondral bone
SM	Synovial membrane
WALANT	Wide-awake local anesthesia no tourniquet

References

1. Brandt, K.D.; Radin, E.L.; Dieppe, P.A.; van de Putte, L. Yet more evidence that osteoarthritis is not a cartilage disease. *Ann. Rheum. Dis.* **2006**, *65*, 1261–1264. [[CrossRef](#)]
2. Radin, E.L.; Rose, R.M. Role of subchondral bone in the initiation and progression of cartilage damage. *Clin. Orthop. Relat. Res.* **1986**, *213*, 34–40. [[CrossRef](#)]
3. Scanzello, C.R.; Goldring, S.R. The role of synovitis in osteoarthritis pathogenesis. *Bone* **2012**, *51*, 249–257. [[CrossRef](#)]
4. Suri, S.; Walsh, D.A. Osteochondral alterations in osteoarthritis. *Bone* **2012**, *51*, 204–211. [[CrossRef](#)] [[PubMed](#)]
5. Radin, E.L. The physiology and degeneration of joints. *Semin. Arthritis Rheum.* **1972**, *2*, 245–257. [[CrossRef](#)] [[PubMed](#)]
6. Zhu, S.; Zhu, J.; Zhen, G.; Hu, Y.; An, S.; Li, Y.; Zheng, Q.; Chen, Z.; Yang, Y.; Wan, M.; et al. Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. *J. Clin. Investig.* **2019**, *129*, 1076–1093. [[CrossRef](#)] [[PubMed](#)]
7. Sun, Q.; Li, G.; Liu, D.; Xie, W.; Xiao, W.; Li, Y.; Cai, M. Peripheral nerves in the tibial subchondral bone: The role of pain and homeostasis in osteoarthritis. *Bone Jt. Res.* **2022**, *11*, 439–452. [[CrossRef](#)]

8. Sharma, L. Osteoarthritis of the Knee. *N. Engl. J. Med.* **2021**, *384*, 51–59. [[CrossRef](#)]
9. Morgan, M.; Thai, J.; Nazemian, V.; Song, R.; Ivanusic, J.J. Changes to the activity and sensitivity of nerves innervating subchondral bone contribute to pain in late-stage osteoarthritis. *Pain* **2022**, *163*, 390–402. [[CrossRef](#)]
10. Buchheit, T.; Huh, Y.; Maixner, W.; Cheng, J.; Ji, R.R. Neuroimmune modulation of pain and regenerative pain medicine. *J. Clin. Investig.* **2020**, *130*, 2164–2176. [[CrossRef](#)]
11. Sanchez, M.; Fiz, N.; Guadilla, J.; Padilla, S.; Anitua, E.; Sanchez, P.; Delgado, D. Intraosseous infiltration of platelet-rich plasma for severe knee osteoarthritis. *Arthrosc. Tech.* **2014**, *3*, e713–e717. [[CrossRef](#)] [[PubMed](#)]
12. Sanchez, M.; Delgado, D.; Sanchez, P.; Muinos-Lopez, E.; Paiva, B.; Granero-Molto, F.; Prosper, F.; Pompei, O.; Perez, J.C.; Azofra, J.; et al. Combination of Intra-Articular and Intraosseous Injections of Platelet Rich Plasma for Severe Knee Osteoarthritis: A Pilot Study. *Biomed. Res. Int.* **2016**, *2016*, 4868613. [[CrossRef](#)] [[PubMed](#)]
13. Anitua, E.; Padilla, S.; Prado, R.; Alkhraisat, M.H. Platelet-rich plasma: Are the obtaining methods, classification and clinical outcome always connected? *Regen. Med.* **2022**, *17*, 887–890. [[CrossRef](#)] [[PubMed](#)]
14. Anitua, E.; Nurden, P.; Prado, R.; Nurden, A.T.; Padilla, S. Autologous fibrin scaffolds: When platelet- and plasma-derived biomolecules meet fibrin. *Biomaterials* **2019**, *192*, 440–460. [[CrossRef](#)]
15. van Buul, G.M.; Koevoet, W.L.; Kops, N.; Bos, P.K.; Verhaar, J.A.; Weinans, H.; Bernsen, M.R.; van Osch, G.J. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. *Am. J. Sports Med.* **2011**, *39*, 2362–2370. [[CrossRef](#)]
16. Bendinelli, P.; Matteucci, E.; Dogliotti, G.; Corsi, M.M.; Banfi, G.; Maroni, P.; Desiderio, M.A. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: Mechanisms of NF-kappaB inhibition via HGF. *J. Cell. Physiol.* **2010**, *225*, 757–766. [[CrossRef](#)]
17. Montaseri, A.; Busch, F.; Mobasher, A.; Buhmann, C.; Aldinger, C.; Rad, J.S.; Shakibaei, M. IGF-1 and PDGF-bb suppress IL-1 β -induced cartilage degradation through down-regulation of NF- κ B signaling: Involvement of Src/PI-3K/AKT pathway. *PLoS ONE* **2011**, *6*, e28663. [[CrossRef](#)]
18. Xu, Z.; Yin, W.; Zhang, Y.; Qi, X.; Chen, Y.; Xie, X.; Zhang, C. Comparative evaluation of leukocyte- and platelet-rich plasma and pure platelet-rich plasma for cartilage regeneration. *Sci. Rep.* **2017**, *7*, 43301. [[CrossRef](#)]
19. Yin, W.; Xu, H.; Sheng, J.; Xu, Z.; Xie, X.; Zhang, C. Comparative evaluation of the effects of platelet-rich plasma formulations on extracellular matrix formation and the NF-kappaB signaling pathway in human articular chondrocytes. *Mol. Med. Rep.* **2017**, *15*, 2940–2948. [[CrossRef](#)]
20. Arra, M.; Abu-Amer, Y. Cross-talk of inflammation and chondrocyte intracellular metabolism in osteoarthritis. *Osteoarthr. Cartil.* **2023**, in press. [[CrossRef](#)]
21. Arra, M.; Swarnkar, G.; Alippe, Y.; Mbalaviele, G.; Abu-Amer, Y. I κ B- ζ signaling promotes chondrocyte inflammatory phenotype, senescence, and erosive joint pathology. *Bone Res.* **2022**, *10*, 12. [[CrossRef](#)] [[PubMed](#)]
22. Sanchez, M.; Delgado, D.; Pompei, O.; Perez, J.C.; Sanchez, P.; Garate, A.; Bilbao, A.M.; Fiz, N.; Padilla, S. Treating Severe Knee Osteoarthritis with Combination of Intra-Osseous and Intra-Articular Infiltrations of Platelet-Rich Plasma: An Observational Study. *Cartilage* **2019**, *10*, 245–253. [[CrossRef](#)] [[PubMed](#)]
23. Sanchez, M.; Jorquera, C.; Sanchez, P.; Beitia, M.; Garcia-Cano, B.; Guadilla, J.; Delgado, D. Platelet-rich plasma injections delay the need for knee arthroplasty: A retrospective study and survival analysis. *Int. Orthop.* **2021**, *45*, 401–410. [[CrossRef](#)] [[PubMed](#)]
24. Sanchez, M.; Jorquera, C.; de Dicastillo, L.L.; Fiz, N.; Knorr, J.; Beitia, M.; Aizpurua, B.; Azofra, J.; Delgado, D. Real-world evidence to assess the effectiveness of platelet-rich plasma in the treatment of knee degenerative pathology: A prospective observational study. *Ther. Adv. Musculoskelet. Dis.* **2022**, *14*, 1759720X221100304. [[CrossRef](#)] [[PubMed](#)]
25. Su, K.; Bai, Y.; Wang, J.; Zhang, H.; Liu, H.; Ma, S. Comparison of hyaluronic acid and PRP intra-articular injection with combined intra-articular and intraosseous PRP injections to treat patients with knee osteoarthritis. *Clin. Rheumatol.* **2018**, *37*, 1341–1350. [[CrossRef](#)]
26. Rios Luna, A.; Fahandezh-Saddi Diaz, H.; Villanueva Martinez, M.; Prado, R.; Padilla, S.; Anitua, E. Office-Based Intraosseous Infiltrations of PRGF in Knee Osteoarthritis: Description of Technique. *Arthrosc. Tech.* **2022**, *11*, e917–e921. [[CrossRef](#)]
27. von Elm, E.; Altman, D.G.; Egger, M.; Pocock, S.J.; Gøtzsche, P.C.; Vandenbroucke, J.P.; Initiative, S. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Lancet* **2007**, *370*, 1453–1457. [[CrossRef](#)]
28. Association, W.M. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* **2013**, *310*, 2191–2194.
29. Kon, E.; Di Matteo, B.; Delgado, D.; Cole, B.J.; Dorotei, A.; Drago, J.L.; Filardo, G.; Fortier, L.A.; Giuffrida, A.; Jo, C.H.; et al. Platelet-rich plasma for the treatment of knee osteoarthritis: An expert opinion and proposal for a novel classification and coding system. *Expert Opin. Biol. Ther.* **2020**, *20*, 1447–1460. [[CrossRef](#)]
30. Roos, E.M.; Roos, H.P.; Lohmander, L.S.; Ekdahl, C.; Beynon, B.D. Knee Injury and Osteoarthritis Outcome Score (KOOS)—development of a self-administered outcome measure. *J. Orthop. Sports Phys. Ther.* **1998**, *28*, 88–96. [[CrossRef](#)]
31. Krzywinski, M.; Altman, N. Visualizing samples with box plots. *Nat. Methods* **2014**, *11*, 119–120. [[CrossRef](#)] [[PubMed](#)]
32. Vaquerizo, V.; Padilla, S.; Aguirre, J.J.; Begona, L.; Orive, G.; Anitua, E. Two cycles of plasma rich in growth factors (PRGF-Endoret) intra-articular injections improve stiffness and activities of daily living but not pain compared to one cycle on patients with symptomatic knee osteoarthritis. *Knee Surg. Sports Traumatol. Arthrosc.* **2018**, *26*, 2615–2621. [[CrossRef](#)] [[PubMed](#)]

33. Vaquerizo, V.; Plasencia, M.A.; Arribas, I.; Seijas, R.; Padilla, S.; Orive, G.; Anitua, E. Comparison of Intra-Articular Injections of Plasma Rich in Growth Factors (PRGF-Endoret) Versus Durolane Hyaluronic Acid in the Treatment of Patients With Symptomatic Osteoarthritis: A Randomized Controlled Trial. *Arthroscopy* **2013**, *29*, 1635–1643. [[CrossRef](#)] [[PubMed](#)]
34. Sanchez, M.; Fiz, N.; Azofra, J.; Usabiaga, J.; Aduriz Recalde, E.; Garcia Gutierrez, A.; Albillos, J.; Garate, R.; Aguirre, J.J.; Padilla, S.; et al. A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. *Arthroscopy* **2012**, *28*, 1070–1078. [[CrossRef](#)] [[PubMed](#)]
35. Wang-Saegusa, A.; Cugat, R.; Ares, O.; Seijas, R.; Cusco, X.; Garcia-Balletbo, M. Infiltration of plasma rich in growth factors for osteoarthritis of the knee short-term effects on function and quality of life. *Arch. Orthop. Trauma Surg.* **2011**, *131*, 311–317. [[CrossRef](#)]
36. Lychagin, A.; Lipina, M.; Garkavi, A.; Islaieh, O.; Timashev, P.; Ashmore, K.; Kon, E. Intraosseous injections of platelet rich plasma for knee bone marrow lesions treatment: One year follow-up. *Int. Orthop.* **2021**, *45*, 355–363. [[CrossRef](#)]
37. Sundaram, K.; Vargas-Hernandez, J.S.; Sanchez, T.R.; Moreu, N.M.; Mont, M.A.; Higuera, C.A.; Piuze, N.S. Are Subchondral Intraosseous Injections Effective and Safe for the Treatment of Knee Osteoarthritis? A Systematic Review. *J. Knee Surg.* **2019**, *32*, 1046–1057. [[CrossRef](#)]
38. Belk, J.W.; Kraeutler, M.J.; Houck, D.A.; Goodrich, J.A.; Dragoo, J.L.; McCarty, E.C. Platelet-Rich Plasma Versus Hyaluronic Acid for Knee Osteoarthritis: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Am. J. Sports Med.* **2021**, *49*, 249–260. [[CrossRef](#)]
39. Belk, J.W.; Lim, J.J.; Keeter, C.; McCulloch, P.C.; Houck, D.A.; McCarty, E.C.; Frank, R.M.; Kraeutler, M.J. Patients With Knee Osteoarthritis Who Receive Platelet-Rich Plasma or Bone Marrow Aspirate Concentrate Injections Have Better Outcomes Than Patients Who Receive Hyaluronic Acid: Systematic Review and Meta-analysis. *Arthroscopy* **2023**, *39*, 1714–1734. [[CrossRef](#)]
40. Evans, C.H.; Kraus, V.B.; Setton, L.A. Progress in intra-articular therapy. *Nat. Rev. Rheumatol.* **2014**, *10*, 11–22. [[CrossRef](#)]
41. Kang, M.L.; Im, G.I. Drug delivery systems for intra-articular treatment of osteoarthritis. *Expert Opin. Drug Deliv.* **2014**, *11*, 269–282. [[CrossRef](#)] [[PubMed](#)]
42. Anitua, E.; Zalduendo, M.M.; Prado, R.; Alkhraisat, M.H.; Orive, G. Morphogen and proinflammatory cytokine release kinetics from PRGF-Endoret fibrin scaffolds: Evaluation of the effect of leukocyte inclusion. *J. Biomed. Mater. Res. A* **2015**, *103*, 1011–1020. [[CrossRef](#)] [[PubMed](#)]
43. Martino, M.M.; Briquez, P.S.; Guc, E.; Tortelli, F.; Kilarski, W.W.; Metzger, S.; Rice, J.J.; Kuhn, G.A.; Muller, R.; Swartz, M.A.; et al. Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. *Science* **2014**, *343*, 885–888. [[CrossRef](#)] [[PubMed](#)]
44. Sanchez, M.; Anitua, E.; Delgado, D.; Sanchez, P.; Prado, R.; Goiriena, J.J.; Prosper, F.; Orive, G.; Padilla, S. A new strategy to tackle severe knee osteoarthritis: Combination of intra-articular and intraosseous injections of Platelet Rich Plasma. *Expert Opin. Biol. Ther.* **2016**, *16*, 627–643. [[CrossRef](#)]
45. Karsdal, M.A.; Bay-Jensen, A.C.; Lories, R.J.; Abramson, S.; Spector, T.; Pastoureau, P.; Christiansen, C.; Attur, M.; Henriksen, K.; Goldring, S.R.; et al. The coupling of bone and cartilage turnover in osteoarthritis: Opportunities for bone antiresorptives and anabolics as potential treatments? *Ann. Rheum. Dis.* **2014**, *73*, 336–348. [[CrossRef](#)]
46. Pan, J.; Wang, B.; Li, W.; Zhou, X.; Scherr, T.; Yang, Y.; Price, C.; Wang, L. Elevated cross-talk between subchondral bone and cartilage in osteoarthritic joints. *Bone* **2012**, *51*, 212–217. [[CrossRef](#)]
47. Taheri, S.; Yoshida, T.; Böker, K.O.; Foerster, R.H.; Jochim, L.; Flux, A.L.; Grosskopf, B.; Hawellek, T.; Lehmann, W.; Schilling, A.F. Changes of the subchondral bone microchannel network in early osteoarthritis. *Osteoarthr. Cartil.* **2023**, *31*, 49–59. [[CrossRef](#)]
48. Campbell, T.M.; Churchman, S.M.; Gomez, A.; McGonagle, D.; Conaghan, P.G.; Ponchel, F.; Jones, E. Mesenchymal Stem Cell Alterations in Bone Marrow Lesions in Patients With Hip Osteoarthritis. *Arthritis Rheumatol.* **2016**, *68*, 1648–1659. [[CrossRef](#)]
49. Jeon, O.H.; David, N.; Campisi, J.; Elisseeff, J.H. Senescent cells and osteoarthritis: A painful connection. *J. Clin. Investig.* **2018**, *128*, 1229–1237. [[CrossRef](#)]
50. Muinos-Lopez, E.; Delgado, D.; Sanchez, P.; Paiva, B.; Anitua, E.; Fiz, N.; Aizpurua, B.; Guadilla, J.; Padilla, S.; Granero-Molto, F.; et al. Modulation of Synovial Fluid-Derived Mesenchymal Stem Cells by Intra-Articular and Intraosseous Platelet Rich Plasma Administration. *Stem Cells Int.* **2016**, *2016*, 1247950. [[CrossRef](#)]
51. Anitua, E.; Sanchez, M.; Aguirre, J.J.; Prado, R.; Padilla, S.; Orive, G. Efficacy and safety of plasma rich in growth factors intra-articular infiltrations in the treatment of knee osteoarthritis. *Arthroscopy* **2014**, *30*, 1006–1017. [[CrossRef](#)]
52. Fiz, N.; Delgado, D.; Garate, A.; Sanchez, P.; Oraa, J.; Bilbao, A.M.; Guadilla, J.; Sanchez, M. Intraosseous infiltrations of Platelet-Rich Plasma for severe hip osteoarthritis: A pilot study. *J. Clin. Orthop. Trauma* **2020**, *11*, S585–S590. [[CrossRef](#)]
53. Ilas, D.C.; Churchman, S.M.; McGonagle, D.; Jones, E. Targeting subchondral bone mesenchymal stem cell activities for intrinsic joint repair in osteoarthritis. *Future Sci. OA* **2017**, *3*, Fso228. [[CrossRef](#)] [[PubMed](#)]
54. Ganguly, P.; Fiz, N.; Beitia, M.; Owston, H.E.; Delgado, D.; Jones, E.; Sanchez, M. Effect of Combined Intraosseous and Intraarticular Infiltrations of Autologous Platelet-Rich Plasma on Subchondral Bone Marrow Mesenchymal Stromal Cells from Patients with Hip Osteoarthritis. *J. Clin. Med.* **2022**, *11*, 3891. [[CrossRef](#)] [[PubMed](#)]
55. Liu, H.Y.; Wu, A.T.; Tsai, C.Y.; Chou, K.R.; Zeng, R.; Wang, M.F.; Chang, W.C.; Hwang, S.M.; Su, C.H.; Deng, W.P. The balance between adipogenesis and osteogenesis in bone regeneration by platelet-rich plasma for age-related osteoporosis. *Biomaterials* **2011**, *32*, 6773–6780. [[CrossRef](#)]

56. Zhen, G.; Cao, X. Targeting TGFbeta signaling in subchondral bone and articular cartilage homeostasis. *Trends Pharmacol. Sci.* **2014**, *35*, 227–236. [[CrossRef](#)]
57. Zhu, X.; Chan, Y.T.; Yung, P.S.H.; Tuan, R.S.; Jiang, Y. Subchondral Bone Remodeling: A Therapeutic Target for Osteoarthritis. *Front. Cell Dev. Biol.* **2020**, *8*, 607764. [[CrossRef](#)]
58. Dakin, S.G.; Coles, M.; Sherlock, J.P.; Powrie, F.; Carr, A.J.; Buckley, C.D. Pathogenic stromal cells as therapeutic targets in joint inflammation. *Nat. Rev. Rheumatol.* **2018**, *14*, 714–726. [[CrossRef](#)]
59. Liu, H.Y.; Huang, C.F.; Lin, T.C.; Tsai, C.Y.; Tina Chen, S.Y.; Liu, A.; Chen, W.H.; Wei, H.J.; Wang, M.F.; Williams, D.F.; et al. Delayed animal aging through the recovery of stem cell senescence by platelet rich plasma. *Biomaterials* **2014**, *35*, 9767–9776. [[CrossRef](#)] [[PubMed](#)]
60. Kreuz, P.C.; Kruger, J.P.; Metzloff, S.; Freymann, U.; Endres, M.; Pruss, A.; Petersen, W.; Kaps, C. Platelet-Rich Plasma Preparation Types Show Impact on Chondrogenic Differentiation, Migration, and Proliferation of Human Subchondral Mesenchymal Progenitor Cells. *Arthroscopy* **2015**, *31*, 1951–1961. [[CrossRef](#)]
61. Krüger, J.P.; Hondke, S.; Endres, M.; Pruss, A.; Siclari, A.; Kaps, C. Human platelet-rich plasma stimulates migration and chondrogenic differentiation of human subchondral progenitor cells. *J. Orthop. Res.* **2012**, *30*, 845–852. [[CrossRef](#)] [[PubMed](#)]
62. Krüger, J.P.; Ketzmar, A.K.; Endres, M.; Pruss, A.; Siclari, A.; Kaps, C. Human platelet-rich plasma induces chondrogenic differentiation of subchondral progenitor cells in polyglycolic acid-hyaluronan scaffolds. *J. Biomed. Mater. Res. B Appl. Biomater.* **2014**, *102*, 681–692. [[CrossRef](#)] [[PubMed](#)]
63. Kulawig, R.; Krüger, J.P.; Klein, O.; Konthur, Z.; Schütte, H.; Klose, J.; Kaps, C.; Endres, M. Identification of fibronectin as a major factor in human serum to recruit subchondral mesenchymal progenitor cells. *Int. J. Biochem. Cell Biol.* **2013**, *45*, 1410–1418. [[CrossRef](#)] [[PubMed](#)]
64. Anitua, E.; Pelacho, B.; Prado, R.; Aguirre, J.J.; Sanchez, M.; Padilla, S.; Aranguren, X.L.; Abizanda, G.; Collantes, M.; Hernandez, M.; et al. Infiltration of plasma rich in growth factors enhances in vivo angiogenesis and improves reperfusion and tissue remodeling after severe hind limb ischemia. *J. Control. Release* **2015**, *202*, 31–39. [[CrossRef](#)] [[PubMed](#)]
65. Assirelli, E.; Filardo, G.; Mariani, E.; Kon, E.; Roffi, A.; Vaccaro, F.; Marcacci, M.; Facchini, A.; Pulsatelli, L. Effect of two different preparations of platelet-rich plasma on synoviocytes. *Knee Surg. Sports Traumatol. Arthrosc.* **2015**, *23*, 2690–2703. [[CrossRef](#)] [[PubMed](#)]
66. Anitua, E.; Sanchez, M.; Merayo-Llodes, J.; De la Fuente, M.; Muruzabal, F.; Orive, G. Plasma rich in growth factors (PRGF-Endoret) stimulates proliferation and migration of primary keratocytes and conjunctival fibroblasts and inhibits and reverts TGF-beta1-Induced myodifferentiation. *Investig. Ophthalmol. Vis. Sci.* **2011**, *52*, 6066–6073. [[CrossRef](#)]
67. Zhen, G.; Wen, C.; Jia, X.; Li, Y.; Crane, J.L.; Mears, S.C.; Askin, F.B.; Frassica, F.J.; Chang, W.; Yao, J.; et al. Inhibition of TGF-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. *Nat. Med.* **2013**, *19*, 704–712. [[CrossRef](#)]
68. Kwan Tat, S.; Lajeunesse, D.; Pelletier, J.P.; Martel-Pelletier, J. Targeting subchondral bone for treating osteoarthritis: What is the evidence? *Best Pract. Res. Clin. Rheumatol.* **2010**, *24*, 51–70. [[CrossRef](#)]
69. Seidel, M.F.; Herguijuela, M.; Forkert, R.; Otten, U. Nerve growth factor in rheumatic diseases. *Semin. Arthritis Rheum.* **2010**, *40*, 109–126. [[CrossRef](#)]
70. Rajan, P.V.; Ng, M.K.; Klika, A.; Kamath, A.F.; Muschler, G.F.; Higuera, C.A.; Piuizzi, N.S. The Cost-Effectiveness of Platelet-Rich Plasma Injections for Knee Osteoarthritis: A Markov Decision Analysis. *J. Bone Joint Surg. Am.* **2020**, *102*, e104. [[CrossRef](#)]
71. Van Demark, R.E., Jr.; Becker, H.A.; Anderson, M.C.; Smith, V.J.S. Wide-Awake Anesthesia in the In-Office Procedure Room: Lessons Learned. *Hand* **2018**, *13*, 481–485. [[CrossRef](#)] [[PubMed](#)]
72. Bismil, M.; Bismil, Q.; Harding, D.; Harris, P.; Lamyman, E.; Sansby, L. Transition to total one-stop wide-awake hand surgery service-audit: A retrospective review. *JRSM Short Rep.* **2012**, *3*, 23. [[PubMed](#)]
73. Boffa, A.; Salerno, M.; Merli, G.; De Girolamo, L.; Laver, L.; Magalon, J.; Sanchez, M.; Tischer, T.; Filardo, G. Platelet-rich plasma injections induce disease-modifying effects in the treatment of osteoarthritis in animal models. *Knee Surg. Sports Traumatol. Arthrosc.* **2021**, *29*, 4100–4121. [[CrossRef](#)] [[PubMed](#)]
74. Altman, R.D.; Devji, T.; Bhandari, M.; Fierlinger, A.; Niazi, F.; Christensen, R. Clinical benefit of intra-articular saline as a comparator in clinical trials of knee osteoarthritis treatments: A systematic review and meta-analysis of randomized trials. *Semin. Arthritis Rheum.* **2016**, *46*, 151–159. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.