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Scientific Research

Effect of Autologus Platelet-Rich Plasma on IL-6, MMP-3 and MCP-1 Expression in Synoviocytes from Osteoarthritic Patients Knees

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Abstract

Nowadays, some studies reported promising results of platelet-rich plasma (PRP) for the treatment of osteoarthritis (OA). However, the effects of PRP on prevention of osteoarthritis in knee joints have been debated. The present study investigated the effects of PRP on osteoarthritis-related inflammatory cytokines expressed in fibroblast-like synoviocytes (FLS) from osteoarthritic knees. The synovial tissues were harvested from eight osteoarthritic patients who had undertaken total knee arthroplasties (TKAs) and cultured in DMEM containing 10% FBS. Platelet-rich plasma releasate (PRPr) was made by clotting or activation of PRP by citrate. The levels of PDGF-AA and VEGF in PRPr and whole blood were measured with ELISA method. The FLS were isolated and cultured from osteoarthritic knees. The IL-1β stimulated FLS were cultivated with three different conditions (none, 1% and 10% of PRP). To determine the expression of IL-6, MMP-3, and MCP-1, we used reverse transcriptase polymerase chain reaction (RT-PCR). The concentrations of platelet count in PRP were about 7 to 9 times higher than those of whole blood. The levels of PDGF-AA in PRPr were approximately 3 to 4 times higher than those of whole blood. The levels of VEGF in PRPr were also significantly 7 to 18 times higher than those of whole blood. Without induction of the FLS with IL-1β, 1% or 10% PRPr did not reduce expressions of inflammatory proteins (MMP-3, MCP-1), except for IL-6. However, with induction of the FLS with IL-1β, both concentrations (1% and 10%) of PRPr reduced significantly all inflammatory protein expressions (IL-6, MMP-3, MCP-1). PRPr diminished inflammatory IL-1β-mediated effects on human osteoarthritic fibroblast-like synoviocytes. These results suggest that platelet-rich plasma can be a good therapeutic option for the treatment of osteoarthritis.

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Keywords

Platelet-Rich Plasma, Inflammatory Cytokines, Synoviocyte, Osteoarthritis

1. Introduction

Platelet-rich plasma (PRP) is a simple, low-cost, minimally invasive and widely used treatment that provides a natural concentrate of autologous growth factors (GFs) from the blood. A platelet contains the majority of biologically active molecules required for blood coagulation, such as adhesive proteins, coagulation factors, and protease inhibitors. In addition to the factors that coagulate blood, recent studies have found that activated platelets release many kinds of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β1), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF), which are thought to play a key role in the healing process of many tissues [1]-[3].

These growth factors are known to induce biological changes in cell proliferation and matrix metabolism in a variety of connective tissues, and this fascinating regenerative capacity has led to promising findings but also to some controversies in the scientific community [4]-[6].

PRP has been used as an autologous source of the growth factors to induce regeneration of tissues [7]-[10] such as articular chondrocytes [11], intervertebral disc cells [12] as well as bone regeneration in periodontal and maxillofacial surgery [13].

Despite its widespread application in an orthopaedic field, there is a lack of high level studies demonstrating the real efficacy of PRP on inflammatory cytokines such as interleukin-6 (IL-6), matrix metalloproteinase-3 (MMP-3), and monocyte chemotactic protein-1 (MCP-1), all of which are known as potent inflammatory factors in osteoarthritic knees known to bring about the production of destructive proteases with inhibition of production of extracellular matrix formation. We believe that it is important to have *in vitro* studies to clearly prove the real potential of this biological approach in order to guide its clinical use and to avoid an indiscriminate clinical application. We hypothesize that PRPr significantly reduces inflammatory cytokines such as IL-6, MMP-3 and MCP-1 from human osteoarthritic fibroblast-like synoviocytes (FLS), especially when FLS stimulated with IL-1β.

2. Methods

2.1. Patients

This study was approved by the institutional review board of Chonnam National University Hospital, and informed consent was obtained from all patients (CNUHHIRB 2011-32). We recruited eight patients who were undergoing total knee arthroplasty for osteoarthritis of the knee. Patients had a mean age of 64 years (range, 49 - 82). The mean BMI was 27.5 kg/m^2 (range, 24.3 - 30.8). Patients with primary knee OA were included to this study. The exclusion criteria included secondary OA or rheumatoid arthritis (RA). In addition, smokers and the individuals with systemic disease or history of anticoagulant, immunosuppressive, or antibiotic therapy in the last 6 months or the patients who had diabetes mellitus or severe obesity were excluded. The fibroblast-like synoviocyte (FLS) were obtained from intraoperative specimens. IL-1β stimulated or IL-1β unstimulated FLS were incubated under three different conditions (none, 1% and 10% of PRP). After 24 hours, in the six different wells, the level of IL-6, MMP-3 and MCP-1 were measured by reverse transcription polymerase chain reaction (RT-PCR, PrimeScript™, Takara, Japan).

2.2. PRPr Preparation

Thrombo kit® (Korea Melsmon, S. Korea) was used to separate the PRP from blood samples. 20 mL of venous blood samples were obtained from each individual, and 10 mL of every sample was drawn into a vacu-container containing 1 mL of 0.106 M sodium citrate under sterile conditions and centrifuged at 3200 rpm for 10 minutes at 22˚C. We mixed the anticoagulant with the sampled blood and agitated them. The upper plasma fraction and the buffy coat layer were separated, and 22.8 mM $CaCl_2$ 1:10 (v/v) was added to the separated fraction at 37˚C for 1 hr to activate platelets to release growth factors, and to yield the PRP releasate. This was centrifuged at

3200 rpm for 10 minutes, and 3 mL of supernatant was collected. This platelet-rich plasma releasate was subsequently stored in aliquots of 1.5 mL at −80˚C for further experiments. The experiments were begun just after the synovium were harvested. The level of PDGF-AA and VEGF of PRP releasate was measured using ELISA method and compared them with those of whole blood. Platelets in PRPr were counted by using electronic impedance (XE-2100™ Automated Hematology System, Sysmex, USA) and compared them with those of whole blood.

2.3. Cell Stimulation and Treatment

For all of the experiments, synoviocytes were plated in 6-well culture plates and serum starved for 24 hours in DMEM containing 1% FBS to synchronize cells in a non-activating and nonproliferating phase. Synoviocytes were then cultured in DMEM containing 10% FBS and either 1) maintained as unstimulated and untreated controls, 2) stimulated with 1% PRPr without 10 ng/mL of IL-1β, 3) treated with 10% PRPr without 10 ng/mL of IL-1β, 4) maintained with 10 ng/mL of IL-1β without any PRPr, 5) stimulated with 1% PRPr and 10 ng/mL of IL-1β or 6) treated with 10% PRPr and 10 ng/mL of IL-1β for 24 hours. The optimal concentrations of 10 ng/mL of IL-1β, 1% and 10% PRPr in this study were determined according to the results of our preliminary dose-response study.

2.4. Quantification for the Inflammatory Factors Using RT-PCR Analysis

Synoviocytes were lysed after treatment with various combinations listed in the previous section, and total RNA was extracted with Trizol agent (Life Technologies, Rockville, MD, USA) according to the manufacturer's protocol. Total RNA (1000 ng) was used for reverse transcription and subsequent real-time quantitative polymerase chain reaction (PCR) with gene-specific primers. DNase digestion was carried out using DNA-free (Ambion Inc., Austin, TX, USA) following the manufacturer's guidelines. One microgram of total RNA was converted to cDNA using the Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). For each reaction, 4 mL 5× first-strand buffer (50 mM Trise HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 2 mL of 0.1 M dTT, 5 U RNAsin, 500 mM dNTP mix, 200 pmol Oligo-dT, 25 U Superscript II reverse transcriptase and sterile water were added to the RNA to a volume of 20 µL. This reaction was then incubated at 42˚C for 1 hour. The finished cDNA products were stored in aliquots at 80˚C until needed. Relative expression levels of IL-6, MMP-3, and MCP-1 were calculated as a ratio to the average value of the house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The sequences of primers have been previously described [14] (**Table 1**). The reverse transcription-polymerase chain reaction (RT-PCR) products were separated by electrophoresis on agarose gels and stained with ethidium bromide.

2.5. Statistical Analysis

The values were reported as mean and standard deviation (SD). Levels IL-6, MMP-3 and MCP-1 were compared using one-way analysis of variance. Results were considered statistically significant for p-value < 0.05. Statistical analysis was carried out by using the IBM-Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS Inc. Chicago, USA).

3. Results

3.1. Baseline Platelet Concentration and Growth Factor Analyses

Whole blood samples were counted for platelets, white blood cells, and red blood cells from all eight donors. PRP contained 8.51 ± 0.99 times more platelets than whole blood. In PRPr, which is activated PRP with $CaCl_2$, the growth factors, VEGF and PDGF-AA, were abundantly present in samples from all eight donors. PRPr had 8.42 ± 1.93 times more VEGF and 2.96 ± 0.36 times more PDGF-AA compared to whole blood (**Table 2**).

3.2. Effect of PRPr without Stimulation of IL-1β on Inflammatory Cytokines

Without stimulation of IL-1β, IL-6 was diminished by 6.2% (48.48 ± 2.40) in the 1% PRPr-added medium and by 6.8% (48.18 ± 2.95) on the 10% PRPr-added medium compared with the control group (51.69 ± 3.32) with

Table 1. The sequence of primers in GAPDH, IL-6, MMP-3 and MCP-1.

	Forward primer sequences (5'-3')	Reverse primer sequences (3'-5')
GAPDH	GGGCATGAACCATGAGAAGT	GTCTTCTGGGTGGCAGTGAT
IL-6	GACAGCCACTCACCTCTTCA	TTCACCAGGCAAGTCTCCTC
MMP-3	GACAAAGGATACAACAGGGACCAAT	TGAGTGAGTGATAGAGTGGGTACAT
MCP-1	TCTCTCACGCCAGCACTGACC	GAGTGTTCACATAGGCTTCTG

Table 2. Comparison of concentration in platelet and growth factors between whole blood and PRPr.

Donor	PLT concentration, $\times 10^3$/mL			VEGF concentration, $\times 10^3$/mL			PDGF-AA concentration, $\times 10^3$/mL		
	Whole blood	PRP	N-fold concentration	Whole blood	PRP	N-fold concentration	Whole blood	PRP	N-fold concentration
1	212	1515	7.15	0.301	2.402	7.98	0.504	1.391	2.76
2	294	2098	7.14	0.183	2.252	12.30	0.446	1.462	3.28
3	287	2212	7.70	0.296	2.944	9.95	0.579	1.611	2.78
4	196	1836	9.37	0.259	1.442	5.57	0.517	1.442	3.32
5	177	1520	8.58	0.271	2.354	8.69	0.521	1.253	2.40
6	246	2425	9.85	0.192	1.695	8.82	0.495	1.764	3.56
7	256	2419	7.44	0.258	1.864	6.95	0.518	1.444	2.79
8	269	2375	8.83	0.268	1.905	7.10	0.581	1.632	2.81

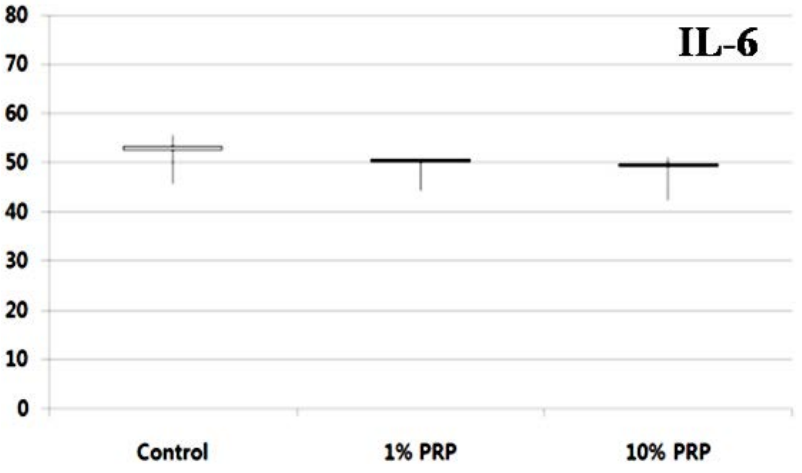
statistical significance (p-value: 0.04, 0.03 each). In terms of MMP-3, without treatment of IL-1β, it was decreased by 2.2% (26.87 ± 3.22) on the 1% PRPr-added medium and by 3.0% (26.68 ± 3.46) on the 10% PRPr-added medium compared with the control group (27.48 ± 3.01) without statistical significance (p-value: 0.71, 0.63 each). For MCP-1, it was diminished by 3.5% (57.92 ± 9.81) on the 1% PRPr-added medium and 4.7% (57.23 ± 10.08) on the 10% PRPr-added medium compared with control group (60.05 ± 10.25) (p-value: 0.67, 0.58 each), which showed no significant difference among the three groups. In summary, without induction of the FLS with IL-1β, 1% or 10% PRP releasate did not show reduced expressions of inflammatory cytokines such as MMP-3 and MCP-1, except for IL-6 (**Figure 1**).

3.3. Effect of PRPr with Stimulation of IL-1β on Inflammatory Cytokines

With stimulation of IL-1β, which activates Nuclear factor kappa B[31] to evoke pathologic OA processes, IL-6 was diminished by 8.2% (49.74 ± 1.74) in the 1% PRPr-added medium and by 8.9% (49.37 ± 1.95) on the 10% PRPr-supplemented medium compared with control group (54.16 ± 1.74) with statistical significance (p-value: 0.03, 0.02 respectively). In terms of MMP-3, with treatment of IL-1β, it was decreased by 8.6% (25.60 ± 0.75) on the 1% PRPr-added medium and 9.6% (25.33 ± 2.80) on the 10% PRPr-treated medium compared with the control group (28.02 ± 2.28) with statistical significance (p-value: 0.03, 0.02 respectively). For MCP-1, under treatment of IL-1β, it was diminished by 6.8% (56.70 ± 2.34) on the 1% PRPr-supplemented medium and 8.9% (55.44 ± 3.98) on the 10% PRPr-treated medium compared with control group (60.84 ± 4.89). We could find significant decreases of cytokines with PRPr (p-value: 0.03, 0.03 respectively). With induction of the FLS with IL-1β, both concentrations (1% and 10%) of PRP releasate significantly reduced all inflammatory cytokine expressions (IL-6, MMP-1 and MMP-3) (**Figure 2**).

4. Discussion

Due to its wide clinical application in the orthopaedic field, a number of reports on PRP have documented clinical results in the treatment of knee degenerative lesions [15]-[25]. In spite of the widespread clinical use of PRP



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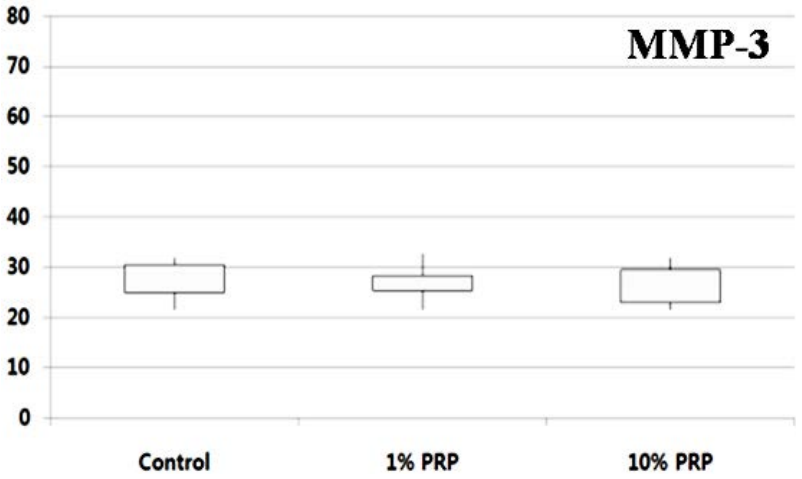

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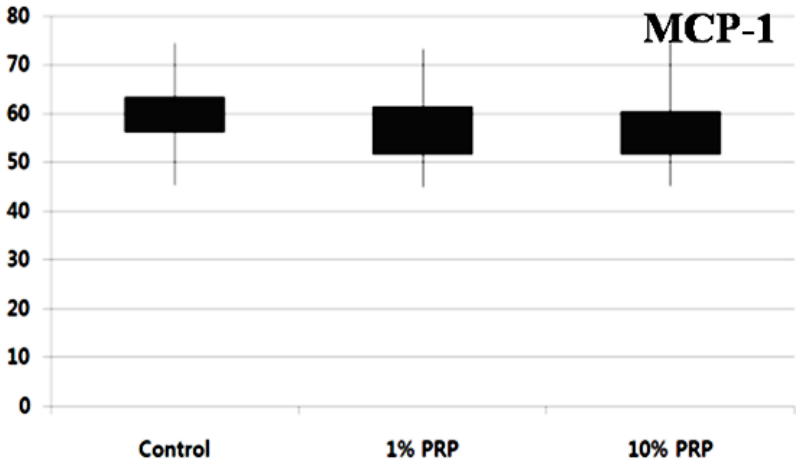
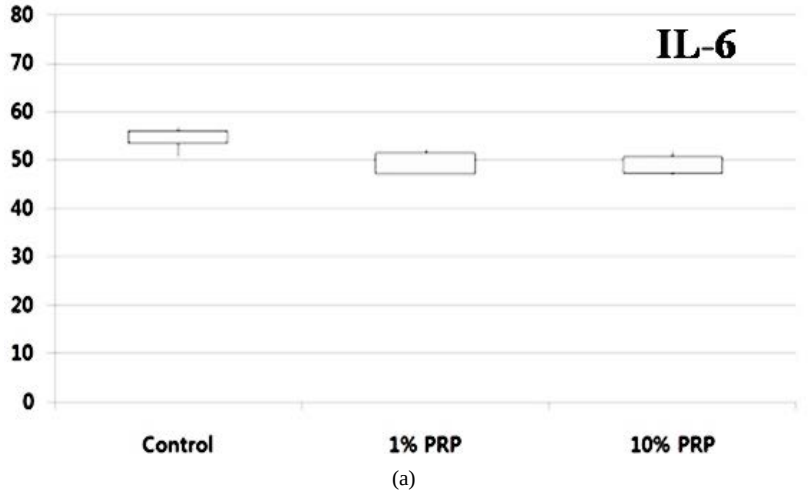
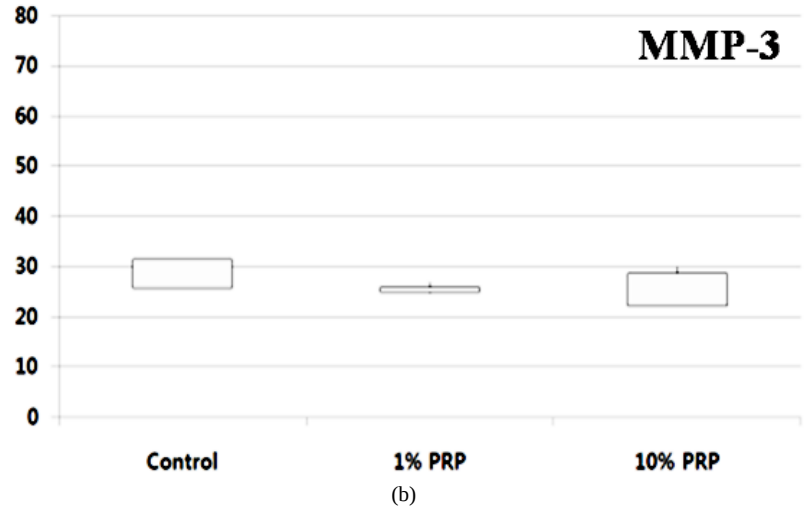


(c)

Figure 1. Effect of PRPr without stimulation of IL-1β on inflammatory cytokines (a) Effect of 1% PRP and 10% PRP on IL-6 without stimulation of IL-1β (p-value: 0.04, 0.03 each); (b) Effect of 1% PRP and 10% PRP on MMP-3 without stimulation of IL-1β (p-value: 0.71, 0.63 each); (c) Effect of 1% PRP and 10% PRP on MCP-1 without stimulation of IL-1β (p-value: 0.67, 0.58 each).



(a)



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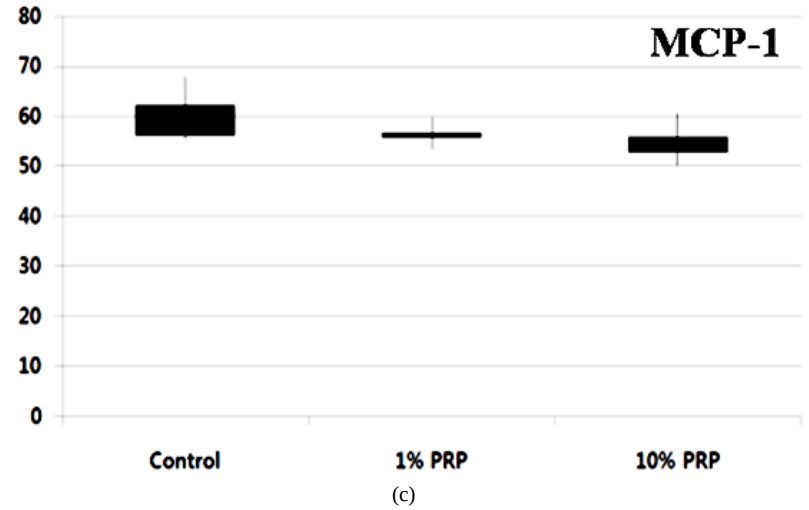

(c)

Figure 2. Effect of PRPr with stimulation of IL-1β on inflammatory cytokines (a) Effect of 1% PRP and 10% PRP on IL-6 with stimulation of IL-1β (p-value: 0.03, 0.02 respectively); (b) Effect of 1% PRP and 10% PRP on MMP-3 with stimulation of IL-1β (p-value: 0.03, 0.02 respectively); (c) Effect of 1% PRP and 10% PRP on MCP-1 with stimulation of IL-1β (p-value: 0.03, 0.03 respectively).

in the treatment of osteoarthritis of knees, there is a wide-ranging debate on clinical efficacy [15]-[25]. In this study, we have evaluated anti-inflammatory effects of PRP with or without IL-1β and have found that, without induction of the FLS with IL-1β, PRPr did not significantly reduce MMP-1 or MMP-3 but did reduce IL-6. However, with induction of the FLS with IL-1β, both concentrations (1% and 10%) of PRPr reduced significantly all inflammatory cytokine expressions (IL-6, MMP-3, MCP-1). It is well known that inflammatory cytokines, such as IL-6, MMP-3 and MCP-1, play an important role in progression of osteoarthritis [15]-[18]. These cytokines have functions in the initiation of inflammatory reaction and in the alteration of extracellular matrix cartilage turnover, which induces pain and promotes joint destruction [25] [26]. There is no doubt that down-regulation those cytokines takes a role in pain relief in osteoarthritic knees [15] [18]-[20].

Many recent studies have reported on the effect of hyaluronic acid on inflammatory cytokines [27]-[29]. However, studies of PRP effects as inflammatory mediators are lacking. Furthermore, there has not been a study comparing anti-inflammatory effect of PRP on synovium in OA knees. In 2012, Stacie *et al*. [30] published a study of current concepts of PRP: "a Milieu of Bioactive Factors" in which they suggested that "Although the effects of many of the proteins in PRP on musculoskeletal tissues are still unknown, they likely contribute to the biologic healing process". In the same year, Gerben *et al*. [31] published a controlled laboratory study on inhibitory PRPr on the inflammatory processes in OA chondrocytes. In their study, PRPr was found to diminish IL-1β-induced inhibition of COL2A1 and ACAN gene expression, and IL-1β-induced increases in ADAMts4 and PTGS2 gene expressions, which implied that PRPr have a favorable effect on chondrocyte behaviors.

5. Conclusion

PRPr significantly reduced inflammatory cytokines (IL-6, MMP-3, MCP-1) in the presence of IL-1β. Although further studies are needed for the application of PRP in clinical settings, these results suggest that platelet-rich plasma can be a great helpful option for the treatment of osteoarthritis.

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Conflicting Interests

The authors declare that they have no competing interests.

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