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# Platelet-Rich Plasma: Formulations, Preparations, Constituents, and Their Effects

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**Platelet-rich plasma (PRP) is a biological treatment option that is increasingly used in sports medicine applications. Outcomes from treating tendon, ligament, muscle, and cartilage injuries with PRP have been variable across many studies, and these differences may be because of the variations in formulations and preparation of PRP. The purpose of this article is to describe the factors that determine the effects of PRP.**


**KEYWORDS** Biologic injections, platelet-rich plasma

## Introduction

Platelet-rich plasma (PRP) has become an increasingly used treatment in the field of sports medicine. The use of autologous blood products as an adjunct to treatment was first pioneered in cardiovascular surgery and in wound-healing applications.[1,2] Oromaxillofacial surgeons then adopted its use, followed by the adoption in both animal and human musculoskeletal applications.

There is much interest in using this autologous treatment option for a wide variety of conditions, including cartilage lesions, tendinopathies, and early osteoarthritis. The numerous formulations and preparation methods of PRP vary widely. A recent meta-analysis reported 14 different indications for treatment and 9 different preparation systems used in clinical studies.[3] When considering using PRP for treatment, analyzing the available literature, or designing investigational trials, it is important to understand the effects of different parameters of PRP preparations that may have on various conditions. The purpose of this article is to provide a broad overview of the differences in formulation and preparation techniques, various delivery methods, and the classification systems of PRP.

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## Classification Systems

Multiple authors have proposed classification systems for the various types of PRP. Dohan Ehrenfest et al[4] described a classification system (Table 1) based on the following 2 factors: cell content, primarily in reference to white blood cells, and a fibrin architecture. With these parameters, PRP can be grouped into 4 different types. Pure PRP (P-PRP), which does not contain leukocytes and has a low-density fibrin network. Leukocyte-rich PRP (L-PRP) has increased concentrations of white blood cells in addition to high concentration of platelets, but also has a low-density fibrin network. Next, pure platelet-rich fibrin is free of leukocytes, but has a high-density fibrin network. Finally, leukocyte- and platelet-rich fibrin combines both increased concentrations of leukocytes and a high-density fibrin network. The preparations with a low-density fibrin network allow for injectable applications, which are more commonly used in orthopedic and sports medicine conditions.[4] Preparations with a high-density fibrin network, including both pure platelet-rich fibrin and leukocyte- and platelet-rich fibrin, allow for a clot with growth factors present in the matrix architecture.[4]

DeLong et al[5] proposed the PAW classification (Table 2) that is based on 3 factors. The 3 components of this system are the number of platelets (P), the activation system (A), and whether white blood cells are present or not (W). Platelet concentration is separated into 4 groups, denoted as P1 through P4. P1 preparations have concentrations at or below baseline values, P2 concentrations are from baseline to 750,000 platelets/μL, P3 range from 750,000 platelets/μL to 1,250,000 platelets/μL, and P4 are concentrations more than 1,250,000 platelets/μL.

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**Table 1** Classification of PRP Types

| Type of Platelet-Rich Plasma | Presence of Leukocytes? | Fibrin Architecture |
|---|---|---|
| Pure platelet-rich plasma (P-PRP) | No | Low density |
| Leukocyte- and platelet-rich plasmas (L-PRP) | Yes | Low density |
| Pure platelet-rich fibrin (P-PRF) | No | High density |
| Leukocyte- and platelet-rich plasma (L-PRF) | Yes | High density |

The usage of an exogenous activator is classified with an *x*. Finally, the leukocyte concentration is grouped as either above (A) or below (B) the baseline value. The neutrophil concentration is similarly grouped as above (α) or below (β) whole blood values. Although the PRP nomenclature remains variable and no single classification system is uniformly used, it is critical for clinicians to know what is in the milieu of PRP that they are injecting into their patients. Only then will the optimal type and timing of PRP injections for each clinical condition be determined.

## Initial Preparation

PRP preparation begins with drawing the patient's peripheral blood. Peripheral blood is composed of 93% red blood cells, 6% platelets, and 1% leukocytes.[6] Care must be taken during the process of drawing blood, as there are parts of the blood draw technique that can influence the final PRP product. For example, premature activation of platelets may occur if a small needle is used to draw blood.[7] Blood for use in PRP preparation should be drawn with 21-gauge or larger needle. Additionally, the speed with which blood is drawn may influence platelet quality, so blood should be aspirated slowly.[8]

After blood is drawn from a patient, it undergoes a centrifugation process to separate the liquid and cellular components. The goal of this spinning process is to concentrate platelets and lower the relative volume of erythrocytes.[6] The first spin is performed at approximately 900 g.[9] The purpose of this step is to separate platelets from the red and white blood cells. Next, a second spin may or may not be employed. This second, faster spin is performed at 1500 g and functions to create a buffy coat and further concentrate the platelets into the same layer as the white blood cells.[9,10]

There are 2 basic methods of PRP preparation, which are plasma-based and buffy coat based.[5] Plasma-based preparations are produced with only the initial slow and short (5 minutes) spin and no second spin. This process leads to isolation of plasma and platelets while reducing leukocytes and erythrocytes from the preparation. The final volume of platelets from this method is usually 2-3 times more than the initial concentrations. Alternatively, a buffy coat–based preparation may be prepared.[5] This method attempts to isolate the maximum level of platelets and does so with a second, high spin speed centrifugation for 10-15 minutes. Leukocytes and erythrocytes remain in the preparation, though the platelet concentrations are higher than those isolated by a plasma-based preparation, at 3-8 times the baseline concentrations.

The materials used for PRP preparation also affect the final product. Polypropylene tubes have been shown to be best for platelet preparation and storage.[11] Tubes made from other materials, including glass and polystyrene, may lead to premature platelet activation or alterations in platelet morphology.[12,13] For these reasons, researchers must note these specifications when describing a PRP protocol, and clinicians should follow these instructions when preparing PRP.

There are multiple commercial systems available to use to prepare PRP. Castillo et al[14] investigated 3 different systems (MTF Cascade, Arteriocyte Magellan, and Biomet GPS III) and showed that there was variability among the PRP preparations with respect to growth factor and leukocyte concentration. There were no significant differences regarding platelet concentration, with a mean 2.18-fold increase in platelet concentration more than the baseline level. The Cascade system produced a 6-fold decrease in the leukocyte concentration, whereas an increase in leukocyte concentration was observed with the Magellan (5-fold) and GPS III (2-fold) systems. No significant differences were measured for transforming growth factor-beta 1 (TGF-β1) concentrations from the products of each of the 3 systems. The vascular endothelial growth factor (VEGF) concentrations for the Cascade PRP were significantly lower than the GPS III PRP ($P = 0.004$), and the platelet-derived growth factor-AB (PDGF-AB) ($P = 0.006$) and PDGF-BB ($P = 0.008$) concentrations in the Cascade PRP were

**Table 2** PAW Classification of Platelet-Rich Plasma

| Elements of Classification System | Representation | Definition |
|---|---|---|
| Platelet concentration | P1 | Platelet concentration ≤ baseline concentration |
| | P2 | Platelet concentration from baseline to 750,000 platelets/μL |
| | P3 | Platelet concentration from 750,000-1,250,000 platelets/μL |
| | P4 | Platelet concentration > 1,250,000 platelets/μL |
| Activator | *x* | Exogenous activator used |
| White blood cell presence | A | Leukocyte concentration above baseline level |
| | B | Leukocyte concentration below baseline level |
| | α | Neutrophil concentration above baseline level |
| | β | Neutrophil concentration below baseline level |

significantly lower than those concentrations in the Magellan PRP. Kushida et al[15] compared PRP produced by 7 different commercial systems (JP200, GLO PRP, Magellan Autologous Platelet Separator System, KYOCERA Medical PRP Kit, SELPHYL, MyCells, and Dr. Shin's THROMBO KIT). The platelet concentration ranged from a 9-fold increase over baseline with the Magellan system to a platelet concentration that was 0.52 times the baseline level with the SELPHYL system. Aside from the low platelet concentration produced by the SELPHYL system, all of the other 6 systems produced platelet concentration at least 3 times higher than the baseline. The PDGF-AB concentration was not significantly different between 5 systems (JP200, GLO PRP, Magellan, KYOCERA, and MyCells), but the other 2 systems (SELPHYL and Dr Shin) produced PRP with significantly lower PDGF-AB. Finally, the cost of these systems ranged from US$50 (JP200) to US$500 (Magellan). The Magellan PRP system was the only common system between these 2 studies, and the platelet concentration ranged from 2.8 times baseline concentrations in Castillo et al[14] to a 9-fold increase over baseline in Kushida et al.[15] These studies demonstrate the variation in PRP based on the system used and highlight the importance in understanding the potential clinical effects from the various components such as platelet and leukocyte concentration in PRP.

There are several patient-specific factors that can be modified to influence the concentration and quality of platelet from an individual patient. A high-fat meal has been shown to increase peripheral platelet concentration in healthy volunteers compared with that during a period of fasting.[16] Circadian rhythms also affect platelet concentration and function, with platelet concentrations increasing the afternoon and platelet activation decreasing from noon to midnight.[17] All of these factors should be recognized when preparing PRP for clinical or research purposes.

## Platelet Concentration

There is also much variability between patients regarding blood composition. The normal circulating concentration of platelets ranges from 150,000-350,000 platelets/μL.[18] Andrade et al[19] demonstrated that the final platelet concentration in PRP was positively correlated with the initial platelet concentration in whole blood ($r = 0.535$). In an evaluation of 6 athletes undergoing Achilles tendon repair with platelet-rich fibrin matrices augmentation, Sánchez et al[20] showed the concentrations of PDGF-AB, TGF-β1, VEGF, hepatocyte growth factor, and epidermal growth factor were all significantly positively correlated with the concentration of platelets. There is a range, however, within which PRP can be effective, and platelet concentrations that are either too low or too high may be ineffective or have adverse clinical effects.

The effect of platelet concentration on outcome was demonstrated by Weibrich et al.[21] In a study on the role of PRP in bone regeneration in rabbits, a platelet concentration of 1,000,000 platelets/μL was found to positively correlate with bone regeneration at 4 weeks. Using PRP of lower concentration (0.5-1.5 times the whole-blood platelet concentration) did not result in enhanced bone regeneration compared with a control group. In contrast, treatment with PRP with platelet concentrations at 6-11 times higher than baseline values showed an inhibitory effect on bone regeneration.

Platelet concentrations from above baseline up to 750,000 platelets/μL (×1-4 baseline) are supported as effective in tissue regeneration by several clinical and preclinical studies. Sánchez et al[20] documented a decrease in the time to return to sport after Achilles tendon repair augmentation with PRP containing platelet concentration of 3 times starting blood concentrations. This study reported earlier recovery of range of motion (7 weeks in the PRP group vs 11 weeks in the control group; $P = 0.025$), and that the athletes with PRP-augmented repair were able to return to sport 7 weeks earlier than athletes treated with tendon repair alone (14 weeks in the PRP group vs 21 weeks in the control group; $P = 0.004$). In an equine study, Torricelli et al[22] found that platelet concentrations of 750,000 platelets/μL (mean = 5.4 times baseline concentration) were optimal for returning race horses to competition after musculoskeletal injuries. Horses that received PRP with platelet concentrations greater than 750,000 platelets/μL returned to competition at 2.8 months, compared with 7.9 months for those that received concentrations less than 750,000 platelets/μL ($P = 0.049$).

High concentrations for platelets are generally defined as being between 750,000 and 1,800,000 platelets/μL or 4-6 times the baseline platelet concentration.[5] Hee et al[23] described improved bone healing for lumbar interbody fusions treated with high-concentration PRP that was 4.89 times the baseline platelet concentration as compared to a historical control group of patients who had undergone interbody fusions without PRP application. There was a 100% fusion rate in single-level fusions and 90% fusion rate for multilevel fusions. The authors noted no pseudarthroses, as compared with a 4% rate in a historic control group. Increasing angiogenesis contributes to healing in meniscal tears, tendon injuries, and other areas of poor vascularity.[25] The angiogenic effects of PRP, as measured through stimulation of proliferation of human umbilical vein endothelial cells, peaked at this platelet concentration with decreasing effectiveness in both lower and higher concentrations of platelets. An in vitro study on endothelial cell proliferation found the best results with a platelet concentration of 1,500,000 platelets/μL.[24] These studies emphasize the role that platelet concentration has on the clinical effects of PRP. This suggests that platelet concentration should be noted and adjusted based on the clinical indication for treatment purposes and in research studies.

## Leukocyte Concentration

Leukocytes are found in the peripheral circulating blood and are a key component of the normal immune system. The grouping of leukocytes includes neutrophils, eosinophils, basophils, lymphocytes, and monocytes.[26] Platelets and leukocytes interact in multiple different complex manners, including leukocytes binding to activated platelets for transmigration, and platelets improving recruitment of leukocytes to areas of inflammation.[27] The presence or absence of

leukocytes from the formulation strongly influences the function of PRP. Depending on the purpose for treatment and the location of injection, leukocytes may have positive or negative effects. Increased leukocyte concentrations are correlated with increased concentrations of inflammatory cytokines, such as interleukin-1β (IL-1β), TNF-, IL-6, and IL-8.[28–31]

There is a risk for muscle damage, if L-PRP is injected for the treatment of an acute muscle injury, primarily owing to neutrophils. Neutrophils are present shortly after muscle injury and help degrade byproducts of muscle damage. The actions of these cells, however, also may cause decreased muscle contractility and direct muscle cell membrane lysis.[32] Dragoo et al[33] demonstrated an increased inflammatory response after the injection of rabbit tendons with L-PRP compared with leukocyte-poor PRP. In the L-PRP group, the tendon structure 5 days after injection was significantly more disrupted compared with the leukocyte-poor PRP. Additionally, the L-PRP group had significantly more fibrosis at 5 days than the leukocyte-poor PRP group. By 14 days, however, there were no observable differences among the groups as all tendons showed evidence of increased cellularity. McCarrel et al[31] reported on the effects of PRP with varying leukocyte concentrations on healing in horse flexor tendons. High concentrations of leukocytes in PRP were associated with increased expression of IL-1β and TNF-α, which are observed in tendinopathy but not normal tendons. These findings suggest that the addition of leukocytes in PRP may be counterproductive when using PRP to treat tendon-based conditions.

The presence of leukocytes may alter the effects of PRP when injected intra-articularly. Filardo et al[34] treated 144 patients with all levels of knee osteoarthritis (Kellgren-Lawrence grades 0-4) with 3 injections of either platelet-rich growth factor, which was absent of leukocytes, or PRP, with 8,300 leukocytes/μL. Both groups showed significant improvement in International Knee Documentation Committee (IKDC) subjective scores and Tegner score, though there were no differences between the 2 in these outcomes. Pain and swelling, however, were significantly more common in the leukocyte-rich group compared with the leukocyte-poor group. Severe pain was observed in 20% of patients with PRP, compared to 7% of patients with platelet-rich growth factor injection ($P = 0.0005$). Swelling was found in 15% of patients with PRP injection vs 4% of patients with platelet-rich growth factor injection ($P = 0.03$).

An in vitro study by Cavallo et al[35] explored the different effects of pure PRP without leukocytes and PRP on osteoarthritic chondrocytes. Both formulations, as well as platelet-poor plasma, were tested in 3 concentrations: 5%, 10%, and 20%. All formulations resulted in increased concentrations of pro-chondrogenic growth factors such as fibroblast growth factor-β and TGF-β1. The preparations also contained, however, factors including VEGF and PDGF-AB/BB that may work in opposition to the anabolic effects of fibroblast growth factor-β and TGF-β1. Chondrocyte cell proliferation was best at 7 days with PRP without leukocytes, whereas hyaluronan secretion was highest after administration of PRP with leukocytes.[36] These differential results highlight the importance of understand the specifics of PRP preparation and show that the ideal formulation will vary based on the clinical indication.

## Activation Method, Carriers, and Additives

Different activators and carriers are used in PRP research and clinical applications with diverse clinical outcomes. PRP can be activated in a number of ways, and there has been debate about the necessity of, and optimal activation method used in clinical practice. As part of the activation pathway, platelets release alpha granules.[37] This process normally occurs whenever platelets come into contact with collagen, usually becuase of vascular injury. This natural process can be exploited in the clinical use of PRP to control the timing of growth-factor release.

The objective of exogenous activation of PRP to generate PRF before injection is to ensure that growth factors are immediately available.[38] Exogenous activation results in a clot that can then be implanted in the desired location. The fibrin matrix of PRF provides a structural framework with embedded growth factors.[4] This may allow for more targeted treatment and is often used in the surgical application of PRP. Bovine thrombin has been used as one of the methods to activate PRP.[39] Autologous thrombin is another option, either with or without calcium chloride. These chemicals function to catalyze the conversion of fibrinogen to fibrin.[40] The use of bovine thrombin, however, is not without disadvantage. This has been associated with hemorrhage, thrombosis, and immune reaction.[41,42] Calcium chloride can be used as a weak exogenous activator of PRP, leading to release of PDGF-AB.[5,43] Patients, however, may experience increased pain becuase of calcium chloride owing to its low pH of 6.3.[5] Endogenous activation relies on the exposure of PRP to collagen or coagulation factors expressed after injection. Harrison et al[44] compared concentrations of TGF-B1, PDGF-AB, and VEGF each day for 7 days after activation of PRP with either thrombin or collagen. The use of thrombin as an activator resulted in the immediate release of growth factors, whereas there was a showed a sustained-release pattern of growth factors over 7 days subsequent to activation with collagen.

Filardo et al[45] applied PRP activated with 10% calcium chloride to patients with chronic degenerative changes of the knee. There were significant improvements observed in the IKDC scores, from 47% of normal at baseline to 67% at 1 year ($P < 0.0005$) and 59% at 2 years ($P = 0.04$). A sheep study on osteochondral defects showed that PRP polymerized with thrombin combined with microfracture was better than unactivated PRP and microfracture or microfracture alone.[46] Treatment with PRP polymerized with thrombin resulted in excellent fill of the defect and the mean stiffness that was similar when compared with normal cartilage. The defect fill was less for unactivated PRP and the biomechanical stiffness was significantly worse ($P = 0.0007$) for the microfracture group alone and the microfracture and unactivated PRP

group. In treatment of focal cartilage injuries, activated PRP in gel form may reduce the risk of diffusion of growth factors through the knee and may function as a scaffold for cartilage repair.

Finally, it is important to consider the pH of any additive and its effect on platelet function. Wahlström et al[47] demonstrated that osteoblastic response to platelets was pH-dependent with more potent growth factors released in a more acidic environment. The pH-based variation in platelet function is also important to recognize when considering the use of local anesthetics. Borg et al[48] evaluated platelet function after incubation with lidocaine, bupivacaine, and tocainide. Lidocaine had the greatest ability to decrease platelet aggregation, and the effects of the local anesthetics on platelet function were time dependent. Porter et al[49] confirmed these findings, reporting that ropivicaine significantly interferes with normal platelet aggregation and coagulation. For these reasons, the necessity of local anesthetics should be carefully considered before using in conjunction with PRP.

## Frequency of Injection

Various studies have employed different frequencies of PRP injections, and the optimal treatment schedule is not defined. Görmeli et al[50] compared 3 injections of PRP, spaced 7 days apart, to 1 PRP injection after 2 injections of saline, or 3 hyaluronic acid injections, or 3 saline injections for patients with knee osteoarthritis. Patients with early arthritis (Kellgren-Lawrence grades I-III) did best with the 3 consecutive PRP injections, as measured by significantly higher EQ-VAS and IKDC-subjective scores at 6 months after treatment and compared with a single-PRP injection or hyaluronic acid injections ($P = 0.001$).[50] Gobbi et al[51] evaluated the efficacy of 2 intra-articular PRP injections to treat 50 patients with knee osteoarthritis. The patients in this study showed significant improvement in symptoms up to 1 year after treatment with these 2 injections. This study, however, did not have a control group to establish the necessity of the second injection. Overall, 2 separate studies showed that 3 PRP injections for patellar tendinopathy can provide effective results at 2-4 years after treatment.[52,53] These studies did not, however, have a comparison group to allow for understanding of the effects of multiple injections or comparison to control. Multiple clinical studies have demonstrated a positive outcome with a single injection of PRP. Peerbooms et al[54] conducted a randomized control trial comparing PRP with corticosteroid injection for lateral epicondylitis. The investigators found significantly improved symptoms in the PRP-treated group, with mean improvement of 53.5% in this group compared with 14.0% improvement in the corticosteroid group ($P < 0.001$). In a follow-up study, these results of a single-PRP injection were maintained out to 2 years after the initial treatment.[55] The ideal injection regimen remains undefined and should be an area of further research.

## Conclusions

PRP is a promising biologic treatment with a wide range of applications in orthopedics and sports medicine. Multiple studies have demonstrated efficacy in a wide variety of challenging conditions, from tendinopathy to osteoarthritis. Understanding the factors that contribute to this variability will allow clinicians and researchers to appropriately use PRP and further define the role of PRP in the treatment of various clinical conditions.

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