



## **Platelet Rich Plasma - Platelet Counts and Application - A Literature Review**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors PK and GK performed the search, interpretation and wrote the manuscript. Author MPSK contributed to analysis and critically revised the manuscript. All authors have discussed and contributed to the final manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Platelet rich plasma (PRP) is a novel method of using plasma concentrated with platelets for wound healing and tissue regeneration. Platelet rich plasma is prepared from the venous blood using a differential centrifugation technique. It involves a separation spin and a concentration spin, yielding platelet rich plasma. PRP products have been classified into 4 types depending upon major cell constituent and fibrin density upon activation. These are as follows: Pure PRP, Leukocyte and PRP, Pure PRF, Leukocyte and PRF. PRF differs from PRP in that it is rich in a high density fibrin network after activation. PRP is abundant in a variety of growth factors such as VEGF, PDGF, TGF, EGF, and Interleukin-1. Literature consists of reports by different authors about the platelet yield of PRP centrifuged by different systems. A number of factors have also been quoted to influence the platelet concentration in platelet rich plasma. Hence, the aim of this review is to discuss the platelet concentration in PRP centrifuged by different systems and to observe for variations if any.

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## 1. INTRODUCTION

Plasma constitutes about 55% of the total blood volume and contains the blood cells suspended in it (red blood cells, white blood cells and platelets) along with clotting factors. Of the blood cells platelets play a highly important role in hemostasis and healing process. This is because of the protein growth factors secreted by platelets that accelerate the process of wound healing. The portion of plasma that is rich in platelets is called Platelet rich plasma (PRP). By definition, a standard platelet concentrate for transfusion that contains  $0.5 \times 10^{11}$  platelets per unit is called a Platelet rich plasma [1]. Depending on the cell content and fibrin architecture, four preparations of platelet rich plasma have been listed as follows [1,2]:

1. Pure Platelet Rich Plasma (P-PRP): These are preparations that are devoid of leukocytes and have a low density fibrin network post activation.
2. Leukocyte and Platelet Rich Plasma (L-PRP): These are rich in leukocytes and have a low density fibrin network post activation.
3. Pure Platelet Rich Fibrin (P-PRF): These are preparations that are devoid of leukocytes and have a high density fibrin network post activation.
4. Leukocyte and Platelet Rich Fibrin (L-PRF): These are rich in leukocytes and have a high density fibrin network post activation.

Platelet rich fibrin (PRF) is called as a second generation PRP and significantly differs from first generation PRP as PRF is devoid of both coagulation and anticoagulation factors. PRF is used increasingly in regenerative dentistry [3].

Platelet Rich plasma (PRP) assumes high importance in the field of regenerative and sports medicine. Initially PRP was used to support hard and soft tissue healing, but currently its use has extended in the treatment of burns, plastic surgeries, during reconstruction in oral and maxillofacial surgeries, in the healing of Achilles tendon rupture in sports medicine, etc, [4]. This can be attributed to the plethora of growth factors present in PRP. These growth factors are not directly present in PRP but are in turn the degranulation products of the intact platelet alpha granules present in PRP [4]. The growth factors

of importance are platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF), interleukin (IL-1), epidermal growth factor (EGF) and fibroblast growth factor (FGF) [5]. Depending on the type of PRP product i.e. whether they're PRP or PRF and whether they're leukocyte rich or leukocyte poor, the application also varies.

Platelet rich plasma (PRP) is prepared by the centrifugation of anticoagulated whole blood that is drawn using a pipette or syringe [4]. There are various commercially available systems that have been developed to prepare PRP in a clinical or laboratory setting. The quality and quantity of platelets in the PRP produced by different systems is till date a point of debate. Various studies have reported on this aspect for different systems.

Previously we have worked on plenty of topics in periodontology [6–19]. Now we are planning to review the difference in platelet counts in platelet rich plasma. Thus, the aim of this review is to discuss the results of various studies on the platelet count in PRP produced by different methods and to check for variations of any.

## 2. METHOD OF PRP PREPARATION

Differential centrifugation of venous blood is the method used for the preparation of PRP. Depending on the type of yield required, there are two basic methods of PRP preparation [2]: 1. PRP method - yields P-PRP 2. Buffy coat method - yields L-PRP.

The PRP method is as follows [2,20]:

The first step is to subject the venous blood collected in tubes with anticoagulants to a separation spin after which three layers are formed: the upper layer consists mostly of platelets and some WBCs, the middle thin layer is buffy coat rich in WBCs and the bottom layer consists mainly of RBCs. The upper layer along with the buffy coat is separated and subjected to concentration spin. After the second spin, the top layer with platelet poor plasma is separated and the platelet rich plasma layer is exposed for use.

In the buffy coat method [2], only a single spin is used, which results in a buffy coat layer that is rich in WBCs (L-PRP) and an underlying RBC layer.

**Table 1. Table showing the results of different studies on centrifugation systems for PRP preparation**

Authors	Year	Comparison groups	Platelet counting method	Result
Weibrich et al. [21]	2001	Curasan PRP kit Vs PCCS PRP system	Automated counter CellDyn 3500	Higher platelet concentration in PCCS PRP system.
Leitner et al. [22]	2006	Vivostat PRF preparation kit, PCCS platelet concentrate collection system, Harvest SmartPReP 2 APC 60 process, and Fibrinet Autologous Fibrin & Platelet system	Manual; using electron microscopy.	Higher platelet count in Harvest SmartPReP 2 APC 60 process
Tamimi et al. [23]	2007	ACE double centrifugation system, Nahita single centrifugation system	Flow cytometry platelet counting	Higher platelet yield in ACE double centrifugation system
Kaux et al [24].	2009	Liege technique, Curasan PRP kit, Plateltex, GPS II and RegenLab	Auto analyser ABX Micros 60	Highest platelet concentration in Plateltex method
Mazzocca et al. [25]	2012	Two single spin processes - PRP <sub>LP</sub> , PRP <sub>HP</sub> One double spin process - PRP <sub>DS</sub>	Coulter automated analyzer	Highest platelet concentration in single spin process -PRP <sub>HP</sub>
Pelletier et al. [4].	2013	Autologous fluid concentrator (APC), Gravitational platelet separation system (GPS)	Roche Sysmex Model XE - 2 100	Highest platelet concentration in GPS system .
Sabarish et al. [20]	2015	Marx R protocol, Okuda K protocol, Landesberg R protocol	Coulter counter Sysmex XT 2000i	Highest platelet yield in Marx R protocol

### 3. FACTORS INFLUENCING VARIATIONS IN PLATELET COUNT OF PRP

Table 1 reveals that over the years a number of studies have been conducted to determine the platelet count in PRP using different systems with each yielding different results. But most of the studies have pointed out a common increase in the platelet concentration of PRP than whole blood. Andrade et al. [26], in their study have confirmed the influence of whole blood platelet concentration on the PRP platelet concentration. Weibrich et al. [21] has revealed that a gender based difference was observed in the platelet concentration of whole blood. A similar variation has been reported in the study by Mazzocca et al. [25], according to which intra-individual variations were observed with

repetitive blood draws regardless of the system used.

In the study by Sabarish et al. [20] three different protocols were used which differed in terms of rpm and time of centrifugation. The Marx protocol was with a separation spin at 1000rpm for 4 minutes followed by a concentration spin at 800 rpm for 9 minutes, whereas the Okuda protocol had a separation spin at 2400 rpm for 10 minutes and concentration spin at 3600 rpm for 15 minutes. Landesberg protocol followed in the same study used a separation spin at 1400 rpm for 10 minutes and concentration spin at 1600 rpm for 10 minutes.

Similarly, a positive correlation between centrifugal force and time and platelet concentration of PRP has been revealed in the study by Arora et al. [27]. The concentration of

platelets was maximum with a force of 440 g at 10 minutes followed by 208 g at 20 minutes. But when time was increased from 10 minutes to 20 minutes at a force of 440 g, the platelet count showed a significant drop.

Dhurat et al., [2] points out additional factors influencing the PRP yield as follows:

1. Draw of blood - The platelet concentration decreases with an increase in the draw time of the blood sample. The author recommends use of a large bore needle (>22) in order to reduce the draw time [2].
2. Centrifugation - The centrifugation force and relative centrifugal field (RCF) are important parameters to be checked prior to centrifugation. The calculation of RCF is influenced by the centrifuge rotor used as different rotors produce different acceleration forces. Centrifuge rate and time are also parameters with considerable influence on platelet concentration [20].
3. Temperature- 12C-16C was the most commonly recommended temperature range for centrifugation by many authors. Cooling helps in obtaining PRP with viable platelets as it retards the activation of platelets.
4. Anticoagulants - The use of EDTA as an anticoagulant is not recommended as it damages the platelet membrane. Instead, sodium citrate and dextrose of sodium citrate are the recommended anticoagulants.

Activation of platelet rich plasma can be done exogenously using thrombin, calcium chloride or by mechanical trauma. Exogenous activation is not required when PRP is used in soft tissues as collagen is a natural activator of PRP. In the above table variations were also observed in the type of platelet counter used. While most studies have resorted to automated analyzer usage some studies have used manual method of platelet counting. Woodell-May et al. [28], have approved the use of automated hematology analyzers for platelet count in PRP, provided the system has proper validation and adequate platelet suspension is provided.

#### 4. APPLICATIONS OF PRP

In recent times, platelet rich plasma has found extensive applications in the field regenerative medicine and dentistry. The basic use of PRP is

to promote wound healing as it is concentrated with growth factors.

#### 4.1 Periodontics

Extensive application of PRP is in the field of Periodontology. It is used in a wide range of minor surgical procedures such as mucogingival surgeries, guided tissue regeneration, bone filling of intrabony defects and regeneration of alveolar ridges [29]. Eppley et al. [30], report that the concentration of platelets and growth factors was many fold higher in PRP than in whole blood. The alpha granules of platelets contain growth factors such as VEGF, PDGF, EGF, IL-1, etc., making it an autologous source of growth factors. When platelets are concentrated and delivered locally as in PRP, stimulation of a more physiological method of healing occurs, rather than delivery of a single growth factor [29]. This significantly adds to the reason for extensive use of PRP in wound healing. PRP has high levels of growth factors particularly TGF beta and PDGF the presence of which increases the adhesion and proliferation of human periodontal ligament cells. Moreover, they also induce the differentiation of these cells to form mineralised tissue thus contributing to periodontal tissue regeneration [31]. This particularly occurs when PRP is used in combination with autologous bone grafts, freeze dried bone allografts and alloplastic bone particles, facilitating bone regeneration. Del Corso et al. [29] have elaborated further on the role of platelet rich plasma in periodontal surgeries. According to their study, when platelet rich plasma was used a covering above filled intrabony defects, it promoted better soft tissue healing, protection of wound, accelerated wound closure and secure bone regeneration. The review by the same authors points out that under GTR membranes, PRP can be used as a sole filling material, giving results comparable to that of conventional bone filling materials. Another concept quoted is to inject platelet rich plasma over the GTR membrane, which promotes better soft tissue healing and prevents wound dehiscence. In such cases, the role of PRP gel is as a true protection membrane.

#### 4.2 Oral Implantology

On the other hand, when PRP was used along with implant surfaces, contradictory results were observed [32]. In this study by Simonpieri PRPs with three different platelet concentrations were tested. It was revealed that the intermediate

platelet concentration group promoted better osseointegration than the other two groups. The effect was meagre at small concentrations, effects increased as the concentration increased and reached a plateau. Beyond this, increasing platelet concentrations promoted inhibitory effect on osseointegration. Also, the bone - implant contact surface area and contact rate was affected on using platelet rich plasma with higher platelet concentration. So, they promoted osseointegration only at optimal levels. At suboptimal levels much effect wasn't observed and at concentrations beyond the optimal range, inhibitory effect on implant osseointegration was observed, as reported by Weibrich et al. [33]. However, it is used for filling of peri implant bone defects, sinus lift procedures during implant placement [32]. The studies also conclude that PRP can be beneficial for intrabony defects, but in case of implants where the biomechanical strength of direct interface is of high importance, the application of platelet rich plasma has no beneficial effects [33,32].

### 4.3 Oral and Maxillofacial Surgery

Reconstruction surgeries are an important part of oral and maxillofacial surgery and are often done post trauma, or post resection of cysts and tumors. Bone grafts form the core of such reconstruction surgeries. With the advent of tissue engineering, a triad of scaffold, cell lines and signalling molecules such as growth factors have gained importance in reconstruction surgery. Since autologous platelet rich plasma is a rich source of several growth factors, and its superior regenerative capacity have accelerated its usage along with any type of bone graft such as autograft, allograft, xenograft or alloplast. This tissue engineering approach has been reported to be highly effective when combined with bone grafts and bone mesenchymal stem cells (BMSCs). In such cases, the platelet rich plasma serves as a cell - supporting medium and matrix for the bone mesenchymal stem cells (BMSCs) [32].

Platelet rich plasma is also used for the treatment of patients on bisphosphonates, anticoagulant or irradiated maxilla [32]. Apart from tissue regeneration in oral and maxillofacial surgery, PRP injection has also been reported to be quite effective in the treatment of Temporomandibular joint osteoarthritis [34]. This is because of the anti-inflammatory, anti-analgesic and chondrogenic capacity of platelet rich plasma. PRP has an inhibitory effect on

inflammatory cytokines and thus provides the anti-inflammatory and anti-analgesic effect when used. Particularly, in cases of TMJ-osteoarthritis, PRP stimulates proteoglycans and collagen production. PRP also causes up-regulation of genes that increase the synthetic capacity of chondrocytes, and prevent their apoptosis. This leads to cartilage synthesis that is useful in the treatment of TMJ osteoarthritis. When used in procedures such as arthrocentesis, it significantly reduces joint pain and clicking sound [35]. PRP when injected into the joint space, increases the production of hyaluronic acid by synoviocytes, increases glycosaminoglycans, joint angiogenesis and also acts as a scaffold for the migration and proliferation of bone marrow derived mesenchymal stromal cells. Since hyaluronic acid acts as a lubricant, there is relief of joint pain and clicking sounds [34,35].

Thus, PRP is used primarily in dentistry, particularly in the field of Periodontology and Oral and Maxillofacial Surgery for regeneration and reconstruction.

## 5. CONCLUSION

Platelet rich plasma gains its use in the field of medicine due to its concentration of thrombocytes and growth factors, that are higher when compared to whole blood. Many different types of systems have been developed for preparation of PRP and the platelet yield also differs based on the system used for centrifugation. The existing literature evidence does not support the use of one particular system for PRP preparation, as different systems have been compared by different authors. For counting platelets, an automated analyzer has been proved to be efficient. A consensus about platelet count in platelet rich plasma cannot be reached because of the use of different types of centrifugation methods. Hence, future research should focus on developing a single validated system for PRP preparation and estimation of growth factors apart from platelet concentration.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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