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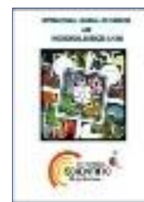

# Comparative Evaluation of Platelet Functional Analysis in Autologous Platelet Concentrates Using Two Different Centrifugation Protocols in Periodontal Regeneration: An *Ex-Vivo* Study

***Arunima Padmakumar Reshma[1*], Sheeja Saji Varghese[2], Nubesh Khan S[3], Lizymol Philipose Pampadykandathil[4], Reghunathan S Preethanath[5], Saranya CV[6]***

*[1]Department of Periodontics and Implantology, PMS College of Dental Sciences and Research, Thiruvananthapuram, Kerala, India, and Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India.*
*[2]Department of Periodontics, Saveetha Dental Collegeand Hospitals,Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India.*
*[3]Department of Preventive Dentistry, College of Dentistry in Ar Rass, Qassim University, Al-Qassim, Kingdom of Saudi Arabia.*
*[4]Division of Dental Products, Department of Biomaterial Sciences and Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.*
*[5]Department of preventive dental sciences, College of dentistry, Jazan University, Saudi Arabia.*
*[6]Department of Biomaterial Sciences and Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.*

**Abstract**

The objective of the current research was to evaluate the impact of different centrifugation protocols on platelets functional attributes by determining Platelet activation, Platelet aggregation and Platelet apoptosis in PRP and PRF blood samples. Blood was collected from 20 healthy volunteer's aged 25 to 35 through antecubital vein venipuncture. The study comprised Group I (PRP) and Group II (PRF), each with 10 samples. Flow cytometry and Annexin V binding assay via flow cytometry assessed platelet activation and apoptosis, respectively, while platelet aggregation was gauged through spectrophotometry. Comparing PRP and PRF revealed substantial differences in platelet function. CD62 expression was significantly higher in PRF than in PRP, indicating heightened activation. Group II (PRF) had remarkable platelet aggregation enhancement compared to Group I (PRP). Apoptotic profiling showed PRF's superior cell viability: Group I (PRP) had higher dead, late apoptotic, and early apoptotic cells, while Group II (PRF) had significantly lower values. PRF also had significantly higher live cells, emphasizing enhanced viability. Quantitative evaluation of platelet function properties offers critical insights into their response to therapeutic interventions and subsequent clinical ramifications. The present study found PRF's potential superiority over PRP, emphasizing its advantageous attributes encompassing enhanced platelet activation, aggregation, and cell viability. These findings contribute to refining platelet concentrate protocols improving wound healing and periodontal regenerative therapies.

**Keywords:**Platelet concentrates, Platelet-rich Plasma, Platelet-rich fibrin, Centrifugation

**Full-length article** **Corresponding Author*, e-mail:*aruniabi@gmail.com*

## 1. Introduction

Periodontal wound healing needs complex interfaces amongst different cells in periodontal tissues, including epithelial cells, periodontal ligament cells, gingival fibroblasts, and osteoblasts. As soon as a wound arises, its disruption of blood vessels triggers the formation of fibrin, the aggregation of platelets, and the release of multiple growth factors from those platelets; these growth factors play a vital role in tissue repair, angiogenesis, inflammation, and immune response. Healing processes of both hard and soft tissues are affected by an array of cellular and molecular events controlled by signaling proteins. Polypeptide growth factors serve as signaling molecules to control every step-in tissue regeneration [1]. Naturally occurring substances found within our bodies -- known as "autologous biomaterials" -- also play a critical role in healing and regeneration processes. Platelets play a pivotal role in blood clotting as well as wound healing, with platelet growth factors providing effective sources of healing

cytokines for clinical applications. Autologous platelet concentrates have become a standard technique in oral and maxillofacial surgery, with various methods developed and utilized for creating concentrated mixtures of fibrin and platelets for topical application. Kinsley coined the term "Platelet-Rich Plasma," or PRP, as an umbrella term to refer to this thrombolytic concentrate in 1954. By 1986, Knighton et al. proposed adding more specific details regarding PRP treatment protocols [2]. Amble Scientific evidence showed the success of platelet concentrates in aiding healing promotion, coining them "platelet-derived wound healing factors" (PDWHF) [3,4].

Autologous products are used in creating first-generation platelet concentrates like PRP for this purpose – to combine fibrin sealant properties with growth factor effects of platelets for maximum healing promotion. This combination provides an effective system to deliver growth factors directly to sites of injury, promoting efficient growth factor delivery. Scientific justification for using such preparations stems from our understanding that growth factors play an essential role in healing hard and soft tissues. These growth factors possess both chemotactic and mitogenic properties that promote and regulate cellular functions involved with tissue healing, regeneration, and proliferation [5]. Blood is collected with anticoagulants before centrifugation (often two-step centrifugation) to remove red blood cells and any unnecessary acellular plasma to collect mostly platelets as the final product. These cells are then suspended in plasma rich in fibrinogen and injected onto the surgical site, often along with calcium chloride and bovine thrombin (to promote polymerization of fibrinogen into fibrin and platelet activation) [6].

For PRP therapy to work effectively, multistep centrifugation, non-autologous anticoagulants, bovine thrombin, or calcium chloride injection is usually necessary as well. PRP therapy primarily seeks to attain high concentrations of platelets and growth factors while eliminating leucocytes from their final composition. However, due to anticoagulants being known to hinder healing processes by preventing coagulation and fibrin clot formation, an alternative platelet concentrate product was eventually created as an answer. Choukroun J et al. developed an innovative form of self-clotted platelet concentrates known as "Platelet-Rich Fibrin (PRF)." Due to strong fibrin gel polymerization, they earned themselves the name "PRF." Platelet-rich fibrin (PRF) is prepared from blood drawn from patients through venipuncture and centrifugation processes that isolate specific components for concentration into layers [7]. This method produces layers enriched with leukocytes, helping reduce postoperative infections while improving primary closure by aiding in the reapproximation of surgical flaps. [8] PRF can significantly contribute to intraoperative hemostasis and mitigate inflammation responses, while its autologous source ensures compatibility by eliminating concerns over allergic or immunologic rejection reactions. Platelet-rich plasma (PRP) requires non-autologous anticoagulants, such as bovine thrombin, to avoid premature clot formation and thus increases susceptibility to infection and allergic manifestations [8].

Furthermore, PRP has a significantly shorter shelf life than PRF, potentially impeding its capacity for prolonged regenerative processes and tissue repair. Absent an anticoagulant, the absence of blood samples activated within minutes by coming in contact with glass tube walls can trigger multiple coagulation cascades, resulting in major platelet activation and subsequent release of coagulation cascades. The accomplishment of thismethod depends completely on how fast blood can be collected and transferred into a centrifuge [9]. This research seeks to conduct an in-depth comparative evaluation of different centrifugation protocols for creating Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF). The current study assessed the differential impacts of various protocols on platelets' functional attributes, with special consideration given to activation dynamics and aggregation tendencies [10]. Furthermore, its primary goal is to identify an efficient strategy for employing platelets for oral wound healing.

## 2. Materials and Methods

### *2.1. Blood Sample Collection and Study Design*

Blood was collected from 20 healthy volunteers aged 25 to 35 through antecubital vein venipuncture. The study comprised Group I (PRP) and Group II (PRF), each with 10 samples. Ethical approval was obtained from Saveetha University's institutional ethical committee by the Helsinki Declaration of 1975 (revised in 2008). Power analysis was conducted using G-Power 3.1.3 software to determine the sample size for a power of 90% and α error of 5%. Inclusion criteria encompassed healthy non-smoking volunteers without systemic medications, while exclusion criteria included subjects with systemic diseases, anticoagulant medication history, or recent antibiotic intake. Licensed and certified professionals performed blood collection.

### *2.2. Preparation of PRP*

Venous whole blood (10 mL) was obtained via antecubital vein venipuncture and collected in sterile centrifuge tubes containing 10% trisodium citrate as an anticoagulant, followed by centrifugation at 1600g for 6 minutes to separate three layers: red blood corpuscles at the bottom, platelet-poor plasma (PPP) on top and platelet-rich plasma (PRP) between. PPP, PRP, and some red blood corpuscles were aspirated using a sterile syringe and transferred into another tube. Further centrifugation separated three layers: red blood corpuscles at the bottom. In comparison, residual red blood corpuscles were aspirated using a sterile syringe for a faster centrifugation process, leaving three layers: red blood corpuscles (bottom), platelet-poor plasma (PPP), and platelet-rich plasma (PRP). Finally, concentrated platelets in suspension were collected after discarding most of PPP [10,11].

### *2.3. Preparation of PRF*

Around 10 mL of blood was collected from each donor via antecubital vein venipuncture and collected into sterile tubes without anticoagulant solutions. PRF was created by centrifuging it at 2700rpm for 12 minutes to produce a fibrin clot composed of RBCs at the bottom and PPP layers on top; the PPP layer was aspirated, yielding PRF [12].

### *2.4. Flow Cytometry Analysis*

Platelet Activation-CD62 Marker Expression: PRP and PRF samples were dispersed into 0.9% NaCl (20:1) before vortexing for 5 seconds to dissolve the supernatant. After discarding, platelets were stained with 1ul of monoclonal mouse anti-human CD62P antibody from Biologicals Canada at a final concentration of 1:100 before analysis within 1 hour using an LSR II Flow Cytometer from Becton. Dickinson in San Diego, CA, USA, where antibody-positive platelets could be identified using fluorescence intensity [13].

### *2.5. Platelet Apoptosis Profile*

The technique for binding Annexin V was used to identify apoptosis. For 24 hours, treated cells were grown and exposed to substances at their LC50 concentrations. Trypsinization was performed on the cells after incubation, and 100 L of the cell suspension was then transferred to other tubes. Each tube received MuseTM Annexin V & Dead Cell Reagent, which was then combined and incubated for 20 minutes. Utilizing the flow cytometry analysis program Muse FCS 3.0 [14].

### *2.6. Determination of Platelet Aggregation using spectrophotometry*

Platelet concentrate samples were transferred into 0.2 mL highly transparent PCR tubes from Nippon Genetics Co., Ltd. (Tokyo, Japan). Platelet aggregation in response to epinephrine was determined spectrophotometrically at 412 nm for 20 minutes. Epinephrine was used at a final concentration of 2.5 x 10 -5 M. PRF, and PPP were diluted with 1X PBS, added with epinephrine, and incubated for 5 minutes [15].

### 2.7. Statistical Analysis

Data analysis utilized SPSS version 20.0 (Chicago, IL, USA), performing unpaired t-tests for group comparisons.

## 3. Results

The mean values for each group are presented as mean ± standard deviation (SD). The degree of in vitro activation, as indicated by CD62 (p-selectin) expression, showed significantly higher mean expression of CD62 in Group II (PRF) (mean ± SD: 97.73 ± 0.60) compared to Group I (PRP) (mean ± SD: 77.76 ± 1.68) (p-value < 0.0001; Figure 1). Regarding platelet aggregation, the mean ± SD value was 34.33 ± 0.51 for PRP and 37.83 ± 0.75 for PRF (p-value < 0.0001; Table 2, Figure 2). The apoptotic profile revealed that in Group I (PRP), the mean ± SD values of dead cells, late apoptotic cells, early apoptotic cells, and live cells were 3.76 ± 0.005, 1.54 ± 0.008, 0.36 ± 0.008, and 94.30 ± 0.008, respectively. For Group II (PRF), the mean ± SD values of dead cells, late apoptotic cells, early apoptotic cells, and live cells were 0.31 ± 0.28, 0.73 ± 0.004, 0.41 ± 0.008, and 98.95 ± 0.87, respectively (p < 0.05). PRF 8 exhibited significantly lower mean values of dead cells, late apoptotic cells, and early apoptotic cells than PRP, indicating improved cell viability in PRF. Additionally, PRF displayed a significantly higher mean value of live cells than PRP, suggesting a higher concentration of viable cells in PRF (Table 3).

## 4. Discussion

The significance of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in regenerative medicine lies in their potential to stimulate tissue neo angiogenesis. This study explored the disparities in centrifugation protocols between PRP and PRF, delving into the consequential impact on crucial platelet functional properties, including platelet activation, aggregation, and apoptotic profile. Notably, this investigation stands as a trailblazer, as it is the first to scrutinize these functional differences between PRP and PRF systematically. The evaluation of platelet concentrates encompassed diverse in vitro techniques, with a particular emphasis on surface receptors through fluorescence-activated flow cytometry [16].The revelations emerging from this study are profound, unveiling substantial discrepancies in platelet activation marker expression between the PRF and PRP groups. CD62P expression, a pivotal marker denoting cell degranulation and platelet activation, surfaced with heightened prominence in the PRF group when juxtaposed with the PRP group. The intricate interplay of shear stress and ADP release during the centrifugation steps appears to underlie this variation in activation patterns, particularly within PRP samples subjected to rigorous centrifugation conditions [16]. Additionally, an intriguing observation comes to light: the study unearths a marked reduction in platelet aggregation within the PRP group compared to PRF (Graph 2, Table 2). This reduction is likely due to the pelleting process following the second centrifugation of the PRP samples. Centrifugation speed and time can disturb and remove larger, active platelets, thereby diminishing platelet aggregation. Studies have shown that larger platelets are more metabolically active and possess greater prothrombotic potential, which may explain the decrease in aggregation with higher centrifuge speeds [16].

Furthermore, the study delves deeper into platelet apoptotic profiles, revealing a compelling finding – PRF demonstrates a conspicuously lower apoptotic profile vis-à-vis PRP. The underlying cause for this decline in apoptotic tendencies within the PRF group is linked to the protective role that activated platelets play against apoptosis, facilitated by releasing platelet- released molecules (PRMs). Activated platelets, wielding the capacity to release distinct agonists, orchestrate a cascade wherein the epidermal growth factor receptor (EGFR) is activated, subsequently triggering DNA-dependent protein [9].kinase (DNA-PK), culminating in the generation of a paracrine signal that effectively curtails apoptosis [16]. Imbued with consistency, these findings resonate harmoniously with previous research that highlights PRF's potential to yield a potent array of growth factors, encompassing the likes of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor (TGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF). [16] This rich ensemble of growth factors occupies pivotal roles in tissue healing, angiogenesis, and cell migration domains. Consonance is also observed in clinical trials investigating the impact of PRF in diverse regenerative procedures – spanning intrabony defects, gingival recession, and regenerative endodontic treatments – with outcomes showcasing promise and potential [17,18].

**Table 1.** Comparison of Platelet activation marker CD62 expression between PRP and PRF.

| Groups | CD62 (MEAN±SD) | p-value |
|---|---|---|
| Group I (PRP) | 77.76±1.68 | 0.0001 |
| Group II (PRF) | 97.73±0.60* | |

**Table 2.** Comparison of mean platelet aggregation between PRP and PRF.

| Groups | Platelet aggregation (MEAN±SD) | p-value |
|---|---|---|
| Group I (PRP) | 34.33±0.51 | 0.0001 |
| Group II (PRF) | 37.83±0.75* | |

(*p<0.05significant compared PRP and PRF)

**Table 3.** Comparison of mean dead cells, late-apoptotic, live apoptotic, and live cells between the groups.

| Groups | Dead cells (MEAN±SD) | Late apoptotic cells (MEAN±SD) | Early apoptotic cells (MEAN±SD) | Live cells (MEAN±SD) |
|---|---|---|---|---|
| Group I (PRP) | 3.76±0.005 | 1.54±0.008 | 0.36±0.008 | 94.30±0.008 |
| Group II (PRF) | 0.31±0.28* | 0.73±0.004* | 0.41±0.008* | 98.95±0.87* |

(*p<0.05 significant compared PRP and PRF)

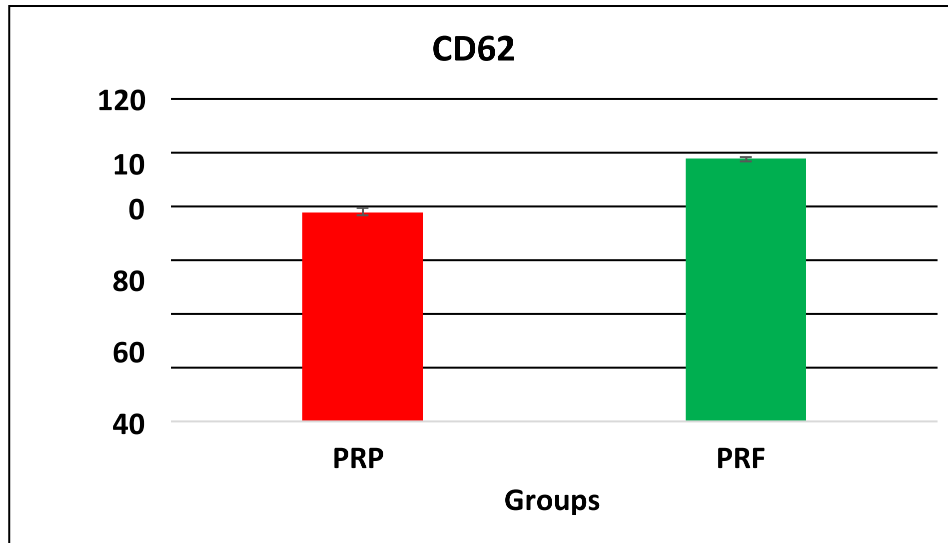


**Figure 1.** Comparison of Platelet activation marker CD62 expression between PRP and PRF

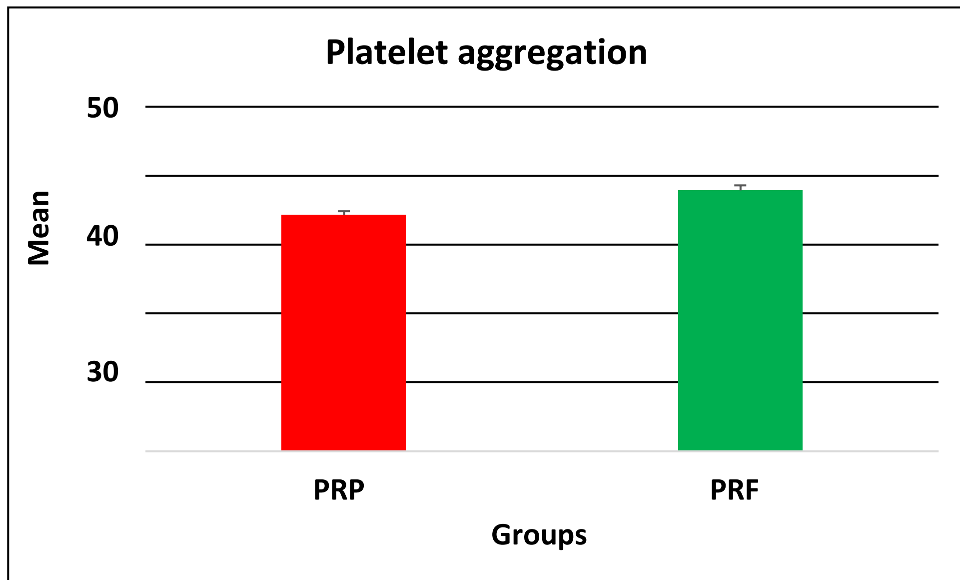


**Figure 2.** Comparison of Platelet aggregation between PRP and PRF

This study casts a powerful spotlight on the functional disparities that distinguish PRP and PRF, accentuating their unique roles in dictating platelet activation, aggregation, and apoptotic processes. Noteworthy is the implication that PRF could confer an advantage when expediting early tissue healing and fostering regeneration. This advantage can be attributed to the intensified platelet activation and mitigated apoptotic tendencies observed within PRF instead of PRP. The implications of these findings are far-reaching, contributing to the ever-expanding body of evidence that champions the applications of platelet concentrates, especially PRF, within the realms of regenerative medicine and dentistry [19,20]. This study acknowledges certain limitations that could have influenced the results and interpretations. The sample size utilized in this investigation might limit the generalizability findings to broader populations. Furthermore, the study's focus on in vitro techniques warrants caution when extrapolating these results to in vivo scenarios. Future study accomplishments could address these shortcomings by conducting larger-scale studies encompassing diverse patient populations and incorporating in vivo models to understand the functional differences between PRP and PRF.

Based on our findings, clinicians should consider the different functional properties of PRP and PRF when choosing platelet concentrates for regenerative therapies. PRF's increased platelet activation and reduced apoptotic tendencies make it an attractive option when rapid tissue healing is desired. PRP's reduced platelet aggregation is preferable when controlled clot formation is desired. Tailoring platelet selection to specific clinical objectives and patient needs would lead to optimal treatment results.

## 5. Conclusions

This research thoroughly investigates the unique properties and roles of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), particularly their effect on tissue regeneration. Through an analysis of centrifugation methods, we reveal significant variations between PRP and PRF regarding activation, aggregation, and apoptotic behavior. PRF exhibits notable advantages over PRP by showing higher platelet activation levels, improved aggregation characteristics, and decreased cell death tendencies compared to its competitors. These findings carry practical applications for platelet concentrate therapies as regenerative therapies. Our recommendations emphasize the significance of selecting platelet concentrates based on specific clinical circumstances and optimizing treatment outcomes for individual patients. Although acknowledging study limitations, we call for future investigations with larger sample sizes and in vivo models to further validate our findings.

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**Conflictsofinterest**

There is no conflict of interest among the authors.

**Authorcontributions**

All authors contributed to the study's conception and design. Dr.ArunimaP.R. performed material preparation and data collection. All other contributing authors supported the data analysis and manuscriptpreparation. All authors read and approved the final manuscript.

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