

Platelet concentration in platelet concentrates and periodontal regeneration-unsrambling the ambiguity

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Abstract

Context: Platelet-rich-plasma (PRP) and Platelet-rich-fibrin (PRF) are extensively used autologous platelet concentrates in periodontal regeneration, and PRF has a better efficacy as compared to PRP. The rationale for this difference has often been attributed to the difference in the structure of the fibrin matrix. However, the effect of concentration of platelets on the regenerative potential of these concentrates is obscure. **Aims:** The study was conducted to evaluate and compare, clinically and radiographically, the efficacy of PRF and PRP in the treatment of periodontal endosseous defects and to assess the effect of platelet concentration on periodontal regeneration. **Materials and Methods:** Twenty intrabony defects were selected and divided into two groups randomly by the coin toss method. Group I received PRP and Group II subjects were treated with PRF. The platelet counts in PRP and PRF were analyzed. Clinical and radiological parameters were assessed at baseline and 3, 6, and 9 months postoperatively. **Statistical Analysis:** Kruskal–Wallis Chi-square test, Wilcoxon signed rank test, *t*-test, and Spearman's rank correlation were used for statistical analysis of data. **Results:** There was statistically significant improvement in all the parameters in the two groups except in relation to gingival recession. There was a statistically significant difference between the platelet count in Group I and Group II ($P = 0.002$). **Conclusion:** PRP and PRF appear to have nearly comparable effects in terms of periodontal regeneration. The concentration of platelets appears to play a paradoxical role in regeneration. The regenerative potential of platelets appears to be optimal within a limited range.

Keywords: Periodontal regeneration, platelet count, platelet-rich-fibrin, platelet-rich-plasma

Introduction

Periodontal regeneration is the absolute restitution of the lost tissues to their original design and function by reiterating the fundamental wound healing events allied with their development. This is considerably assisted by the polypeptide growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF- β), which have been shown to help in cell growth, differentiation, and periodontal regeneration.^[1,2]

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Platelet-rich-plasma (PRP) and platelet-rich-fibrin (PRF) are concentrated suspensions of growth factors and these stimulate healing and regeneration of tissues, including those in the periodontal area.^[3]

Studies on rat long bone to investigate the effect of PRP on the proliferation and differentiation of rat bone marrow cells and to determine an optimal platelet concentration in plasma for osseous tissue engineering showed that mature bone regeneration were more prevalent in the group with the highest concentration of platelets in PRP.^[4]

In a study that analyzed the effect of the platelet count in PRP on bone regeneration *in-vivo*, it was found that, at lower concentrations of platelets, the effect was suboptimal while higher concentrations might have a paradoxically inhibitory effect.^[5]

Studies exist that describe the effects of PRP, PRF on bone regeneration. However, there is a paucity of studies comparing the effects of concentration of platelets in PRP

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and PRF on bone regeneration. This study aims to evaluate and compare the treatment of intrabony defects with PRF, PRP and to analyze the optimal concentration of platelets in PRP and PRF to achieve maximum regeneration.

Materials and Methods

Subjects and study groups

This randomized, longitudinal interventional study involving a total of 11 systemically healthy subjects, contributing to a total of 20 surgical sites, was conducted in our institution. The ethical clearance for the study was obtained from the Ethical Committee and Review Board of the Institution.

Patients aged between 20 and 55 years, who were systemically healthy and had no contraindications for periodontal therapy were included in the study. A patient with a gingival index (GI) score >2.1 and with platelet counts $<200,000/\text{mm}^3$ was considered ineligible for the study. All patients were nonsmokers. Intrabony periodontal defects with a probing pocket depth (PPD) ≥ 5 mm, radiographic defect depth ≥ 3 mm were included in the study.

The total of 20 surgical sites were identified and divided into two groups: Group I and Group II. The coin toss method was used to randomize the patients to receive the treatment options.

The groups were:

- Group I ($n = 10$): Those to be treated with PRP
- Group II ($n = 10$): Those to be treated with PRF.

Clinical and radiographic assessments

Oral hygiene status was assessed using plaque index (PI) (Sillness and Loe [1964]) and GI (Loe and Sillness [1963]). PPD, clinical attachment level (CAL), and gingival recession (GR) were measured to the nearest millimeter with a calibrated periodontal probe using an individual occlusal stent as a reference point for probe placement. Occlusal stents for positioning measuring probes were fabricated with cold-cured acrylic resin on a plaster model obtained from an alginate impression. Measurements were recorded from:

- Stent to cemento-enamel junction (A)
- Stent to gingival margin (B)
- Stent to deepest probing depth at test sites (C).

Calculation of the parameters

PPD = Stent to deepest probing depth at test sites (C) - stent to gingival margin (B).

CAL = Stent to deepest probing depth at test sites (C) - stent to cemento-enamel junction (A).

GR = Stent to gingival margin (B) - Stent to cemento-enamel junction (A).

Intraoral periapical radiographs were taken using the long cone paralleling technique with a radiographic grid in position. The depth of the bone defect was assessed to the closest 0.5 mm on the intraoral periapical radiograph. A horizontal line was drawn projecting from the point on the bone crest designated as "A." The horizontal line was drawn perpendicular to the long axis of the root surface of the tooth associated with the vertical defect and the point of contact of the horizontal line with the root surface was designated as "B." A vertical line was then drawn from "B" to the most coronal level along the root surface where the periodontal ligament space was considered to have a normal width; the point was designated as "C" [Figure 1]. The vertical dimension between "B" and "C" was measured to assess the bone level at the baseline evaluation and was designated as BC0.

Platelet-rich-plasma and platelet-rich-fibrin preparation

PRP was prepared using the following procedure: The left antecubital fossa was swabbed with an alcohol swab and a cuff was used to apply pressure above the fossa. A 10 ml syringe was used to draw 8 ml of blood and immediately transferred into a test tube containing 1.5 ml of citrate anticoagulant solution (anticoagulant citrate dextrose solution). The sample tube was then spun in a standard centrifuge for 10 min at 2400 rpm to produce platelet poor plasma (PPP). The PPP was taken up into a syringe with a long cannula. A second centrifugation (15 min at 3600 rpm) was performed to concentrate the platelets. The second supernatant was also taken up by a long cannula. This was PRP, which was used for the surgical procedure.^[3] At the time of the application, PRP was combined with calcium gluconate to facilitate plasma coagulation.

The PRF was prepared following the protocol developed by Choukroun *et al.*^[6] The patient's blood samples were drawn prior to the surgery following the same procedure as that for PRP preparation. Immediately after the blood draw, the dried monovettes (without anticoagulant) were centrifuged at 3000 rpm for 10 min in the table top centrifuge. A structured

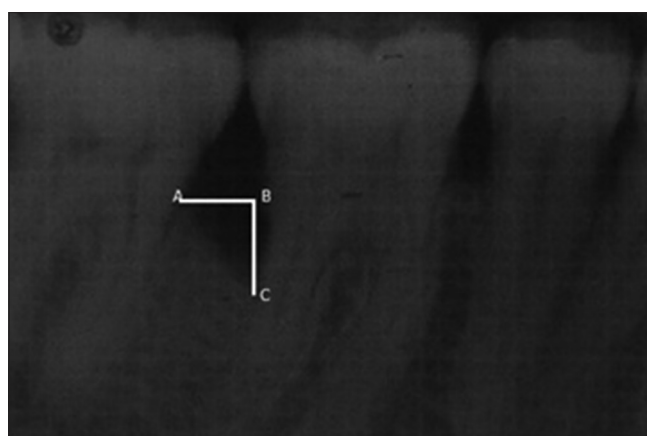


Figure 1: Radiographic assessment

fibrin clot formed in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top PRP and PPP. PRF was separated from red corpuscles at the base, preserving a small red blood cell layer, using a sterile tweezers and scissors just after removal of PPP and then transferred onto a sterile dappen dish.

Platelet count assessment

The number of platelets in the PRP sample were counted manually in the Neubauer chamber after preparing a PRP smear.

Platelet counts were assessed in the donors' whole blood and on the residual serum remaining after PRF preparation; these counts were used to assess the platelet count in the PRF gel prepared.^[7]

Surgical procedure

Local anesthesia was administered, and an intrasulcular incision was placed. Mucoperiosteal buccal and lingual access flaps were then reflected. Granulation tissue was removed to provide full access and visibility to the root surfaces. Any subgingival calculus was removed gently by using hand instruments. Then, PRP or PRF was packed into the defects. Finally, the flaps were replaced and sutured with a 3-0 silk material using interrupted sutures with the direct loop technique. After a healing period of 10 days, the sutures were removed.

Postoperative care

All patients received systemic antibiotic therapy for a period of 5 days postoperatively (amoxicillin 500 mg 3 times per day for 5 days). In addition, all patients were advised to avoid tooth brushing and chewing hard food materials in the surgical areas and to rinse twice daily with a 0.2% solution of chlorhexidine digluconate for 2 weeks. Recall appointments were scheduled every 2nd week during the first 2 months after the surgical procedure, and all patients were recalled once a month for the remaining observation period.

Postsurgical evaluation and review

GI and PI were re-evaluated at 3, 6, and 9 months. PPD, CAL, and GR were also re-evaluated at 3, 6, and 9 months using the previously used acrylic stents to provide a reproducible insertion axis.

Radiographic parameters

The vertical dimension between "B" and "C", measured to assess the depth of the defect at 3, 6, and 9 months, were designated as BC3, BC6, and BC9, respectively. The bone fill at the end of 9 months in each group was obtained by subtracting BC9 from BC0.

Statistical analysis

Statistical analysis was done using the SPSS (SPSS, version 14.0, SPSS, Chicago, IL) software. The intergroup

comparisons were performed using Kruskal–Wallis Chi-square test. The within group comparison was performed using Wilcoxon signed rank test.

Platelet count comparison between the two groups was done using *t*-test. Spearman's rank correlation was used to assess the correlation between platelet count and other parameters at 9 months in Group I and Group II.

Results

A total of 11 subjects contributed to 20 surgical sites. No patients were lost to follow-up. All the subjects returned for clinical and radiographic evaluation at 3, 6, and 9 months. Clinical evaluation of postsurgical healing revealed a good soft tissue response and no adverse complications. Both groups presented similar baseline characteristics in terms of PPD, GR, CAL, PI, and GI.

All patients maintained a good level of oral hygiene and gingival status throughout the recall periods. Intergroup differences were found to be insignificant ($P > 0.05$) in terms of PI and GI.

At 9 months, both the groups presented a significant improvement in terms of PPD reduction and CAL gain [Tables 1 and 2]. The intergroup differences were found to be significant [Table 3]. GR levels had also improved, however, the difference was not statistically significant.

Evaluation of the radiographs indicated that both the treatment modalities resulted in an enhancement of radiodensity at the surgical sites suggestive of bone gain at 9 months in both groups [Tables 1 and 2].

Comparison of platelet count between Group I and Group II and correlation between platelet count and other parameters at 9 months in Group I and Group II (Spearman's rank correlation) are given in Tables 4 and 5. There was a statistically significant difference between the platelet count in Group I and Group II ($P = 0.002$). A negative correlation was found between the platelet count and other parameters in Group I.

Discussion

Periodontal regeneration is a consequence of biological factors that are active regardless of protocol. The production or regeneration of any tissue type is a complex biological process in itself, requiring intricately regulated interactions between cells, locally acting growth factors, systemic hormones and growth factors, and the extracellular matrix components in which these entities interact.^[8,9]

PRP and PRF act as a storage house for growth factors such as TGF- β and PDGF and they have been demonstrated to

Table 1: Comparison of parameters within Group I at different time intervals

Parameter	Time interval	Mean difference	Z [#]	P
Plaque score	Baseline			
	3 months	0.430	-2.232	0.026*
	6 months	0.380	-2.201	0.028*
	9 months	0.780	-2.871	0.004*
GI score	Baseline			
	3 months	0.400	-2.828	0.005*
	6 months	0.400	-2.271	0.023*
	9 months	0.500	-2.887	0.004*
PPD	Baseline			
	3 months	4.750	-2.829	0.005*
	6 months	4.950	-2.829	0.005*
	9 months	6.050	-2.823	0.005*
CAL	Baseline			
	3 months	4.150	-2.829	0.005*
	6 months	4.100	-2.823	0.005*
	9 months	5.100	-2.812	0.005*
GR	Baseline			
	3 months	0.000	0.000	1.000
	6 months	0.000	0.000	1.000
	9 months	0.050	-1.000	0.317
Depth of bone defect	Baseline			
	3 months	3.250	-2.814	0.005*
	6 months	3.550	-2.812	0.005*
	9 months	3.800	-2.809	0.005*

*Significant difference ($P < 0.05$); #Wilcoxon signed rank test statistic. CAL: Clinical attachment level; PPD: Probing pocket depth; GI: Gingival index; GR: Gingival recession

induce healing and regeneration of tissues, including those in the periodontal area.^[3] PRP and PRF are autologous sources of PDGF and TGF- β that is obtained by appropriating and concentrating platelets by gradient density centrifugation. They have been shown to stimulate healing and rejuvenation of tissues including those in the periodontal area.

According to Marx *et al.*, a therapeutic autologous platelet concentrate should present approximately 1 million platelets/ μL in humans, considering that the whole blood contains approximately $200,000 \pm 75,000$ platelets/ μL .^[10] In order to obtain a therapeutic material and in agreement with previous studies, a minimum platelet concentration of 200,000 platelets/ μL was considered vital in this study, and patients with a lower platelet count were excluded.

PPD, CAL, and GR were assessed using a UNC 15 probe which was positioned along the grooves on a customized acrylic stent to provide a reproducible insertion axis for the probe. Similar technique has been implemented in other studies.^[1,11]

Table 2: Comparison of parameters within Group II at different time intervals

Parameter	Time interval	Mean difference	Z [#]	P
Plaque score	Baseline			
	3 months	0.180	-1.786	0.074
	6 months	0.290	-1.866	0.062
	9 months	0.325	-2.111	0.035*
GI score	Baseline			
	3 months	0.278	-1.869	0.062
	6 months	0.212	-1.338	0.181
	9 months	0.266	-1.703	0.089
PPD	Baseline			
	3 months	5.900	-2.825	0.005*
	6 months	6.250	-2.829	0.005*
	9 months	6.500	-2.814	0.005*
CAL	Baseline			
	3 months	5.200	-2.809	0.005*
	6 months	5.450	-2.809	0.005*
	9 months	5.950	-2.812	0.005*
GR	Baseline			
	3 months	0.000	0.000	1.000
	6 months	0.050	-1.000	0.317
	9 months	0.050	-1.000	0.317
Depth of bone defect	Baseline			
	3 months	4.400	-2.823	0.005*
	6 months	4.550	-2.821	0.005*
	9 months	4.850	-2.809	0.005*

*Significant difference ($P < 0.05$); #Wilcoxon signed rank test statistic. CAL: Clinical attachment level; PPD: Probing pocket depth; GI: Gingival index; GR: Gingival recession

Preoperative and postoperative comparability of probing measurements that do not use this standardized method is questionable.^[12]

The PRP preparation has been described by Tözüm and Demiralp.^[3] In this study, calcium gluconate was used as a gelling agent instead of calcium chloride and bovine thrombin. Bovine thrombin has been implicated in the development of antibodies to human clotting factors V, XI, and thrombin resulting in a risk of potentially life-threatening coagulopathies.^[13] Studies have used calcium gluconate as the gelling agent and have found it to be an excellent alternative to the use of calcium chloride and bovine thrombin.^[14,15] The PRF was prepared following the protocol developed by Choukroun *et al.*^[6] This practice has been deemed the most ideal method for PRF preparation for its use in periodontal reconstructive surgeries.

The oral hygiene was maintained satisfactorily in all the subjects until the end of the study period, which could have

Table 3: Comparison of various parameters (PPD, CAL, depth of defect [BL]) between the groups

	Group	Mean	SD	SEM	Kruskal-Wallis χ^2	P	Significant difference between
PPD							
Baseline	I	10.30	1.06	0.33	0.938	0.626	
	II	10.50	1.08	0.34			
3 months	I	5.55	0.80	0.25	10.414	0.005*	1 versus 2
	II	4.60	0.52	0.16			
6 months	I	5.35	0.61	0.19	13.237	0.325	
	II	4.25	0.35	0.11			
9 months	I	4.25	0.50	0.16	14.146	0.001*	1 versus 2
	II	4.00	0.34	0.11			
CAL							
Baseline	I	8.45	1.17	0.37	1.054	0.590	
	II	9.20	1.42	0.45			
3 months	I	4.30	1.06	0.33	11.832	0.003*	1 versus 2
	II	4.00	0.90	0.28			
6 months	I	4.15	0.55	0.17	16.680	<0.001*	1 versus 2
	II	3.75	0.46	0.15			
9 months	I	3.35	0.85	0.27	6.122	0.047*	1 versus 2
	II	3.25	0.91	0.29			
BL							
Baseline	I	8.00	1.33	0.42	5.528	0.063	
	II	9.00	1.18	0.37			
3 months	I	4.75	0.84	0.27	9.457	0.067	
	II	4.60	0.60	0.19			
6 months	I	4.45	0.42	0.13	19.667	<0.001*	1 versus 2
	II	4.25	0.44	0.14			
9 months	I	4.20	0.44	0.14	19.628	0.066	
	II	4.15	0.41	0.13			

PPD: Probing pocket depth; CAL: Clinical attachment level; GR: Gingival recession; BL: Bone level; SD: Standard deviation; SEM: Standard error of mean

Table 4: Comparison of platelet count between Group I and Group II (t-test)

Group	Mean	SD	SEM	Mean difference	t	P
I	1,355,000	184,390.89	58,309.52	342,500	3.666	0.002*
II	1,012,500	98,777.25	31,236.11			

*Significant difference. SD: Standard deviation; SEM: Standard error of mean

been due to the regular reinforcement of oral hygiene. The Hawthorne effect could also have played an important role with regard to oral hygiene maintenance. Hawthorne effect is a form of reactivity whereby subjects improve or modify an aspect of their behavior, which is being experimentally measured, in response to the fact that they know that they are being studied.^[16]

In PRP, the platelet count was measured using a Neubauer chamber. Smear of the platelet concentrate was made, and it was used to assess the platelet count. Similar techniques have been used in other studies.^[17] To assess the platelet count in PRF gel, the platelet counts were assessed in the donors' whole blood and in the residual serum remaining after PRF preparation and then these counts were used to assess the platelet count in the PRF gel prepared. This technique has been used in other studies.^[7]

In Group I, there was an improvement with respect to all clinical and radiographic parameters. Literature has studies which show a similar improvement in clinical and radiographic parameters with the use of PRP.^[18,19] The use of PRP has been shown to have a definite chance of improving the healing in periodontal endosseous defects.

Group II, in which PRF has been used, also showed improvement in all clinical and radiographic parameters. The use of PRF has resulted in the improvement in clinical and radiographic parameters in other studies seen in literature.^[20,21] Bone tissue engineering applications have been seen for PRF, which acts as a suitable scaffold for breeding human periosteal cells *in vitro*.^[1] PRF has been found to have a strong induction effect on osteoblasts also.^[1]

In this study, PRF and PRP have been found to have similar periodontal regenerative effects with PRF showing a slightly superior effect. The comparison between the use of PRP and PRF has been done in a study which showed that there was similar PD reduction, CAL gain, and bone fill at sites treated with PRF or PRP with conventional open-flap debridement. PRF requires less time for preparation and is less technique sensitive.^[22] Hence, currently, PRF is the preferred platelet concentrate for periodontal regenerative procedure.

The fibrin matrix structure of PRF and PRP differ; this might be one of the contributory factors to the superiority of PRF over PRP. The difference between the structures of PRP and PRF is attributable to the gelling mode. PRF has the characteristic of polymerizing naturally and slowly during centrifugation, and there is no addition of any extraneous agent for polymerization. This phase is critical to establish the three-dimensional organization of a fibrin network. In PRF gel, connected junctions are formed between the fibrin fibrillae, and this allows for the establishment of a fine and flexible fibrin network which is able to support cytokines enmeshment and cellular migration. This three-dimensional organization also renders elasticity to the fibrin matrix.^[23]

The superiority of PRF over PRP may also be explained in terms of the difference in platelet count. It was seen in this study that PRP has a greater platelet concentration than PRF, and the difference in platelet count between the PRP and PRF groups was statistically significant. A negative correlation

Table 5: Correlation between platelet count and other parameters at 9 months in Group I and Group II (Spearman's rank correlation)

Parameter	Group I		Group II	
	ρ	<i>P</i>	ρ	<i>P</i>
PPD	-0.566	0.088	0.484	0.156
CAL	-0.529	0.116	0.281	0.432
GR	-	-	-0.233	0.517
BL	-0.225	0.532	-0.370	0.293
Plaque score	-0.370	0.341	0.227	0.528
GI score	0.342	0.334	0.247	0.492

PPD: Probing pocket depth; CAL: Clinical attachment level; GR: Gingival recession; BL: Bone level

was seen between the various parameters measured and the platelet count in the PRP group. The platelet concentration required for a positive effect on bone regeneration appeared to be within a very limited range. Beneficial biological effects seemed to occur when a gel with a platelet concentration of approximately 1000,000/ μ L was used.^[5] The lower concentration of platelets, gave a suboptimal effect while higher concentrations have been shown to have an inhibitory effect.^[5] This might explain the negative correlation between the parameters and platelet count. A study by Hsu *et al.* has shown that the proliferation of oral cells significantly decreased when treated with high concentrations of PRP. Abundant secretion of thrombospondin-1 from concentrated PRP might have contributed to the antiproliferative effect.^[24]

This study is limited by the fact that the exact range of platelet count to achieve the optimal regenerative potential has not been analyzed. A molecular study assessing the factor or factors responsible for the paradoxical effect has to be performed.

Conclusion

PRF and PRP are efficient bone graft substitute materials which have the advantage of being autologous preparations and barely technique sensitive. The use of PRF and PRP also decreases the cost of the regeneration therapy. PRF appears to have a slight advantage over PRP in its value in the management of periodontal endosseous defects. The advantage of PRF over PRP might be due to several reasons ranging from the structure of its fibrin matrix to the platelet count in the preparation. In the study presented in this manuscript, it has been shown that only within a limited range of platelet count does optimal regeneration take place.

Further studies are required to evaluate the exact range of platelet count at which the regenerative potential is at the maximum. Long-term, randomized, controlled clinical trial and histologic and biochemical research will be needed to arrive at a definitive conclusion.

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Conflicts of interest

There are no conflicts of interest.

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