

To Evaluate and Compare the Effect of Platelet Rich Fibrin (PRF) on Crestal/Marginal Bone Loss Around Implants: An In-Vivo Study

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ABSTRACT

Background: The viability of Platelet Rich Fibrin (PRF) on the enhancement of osseous and associated tissue healing has been substantiated well in literature. However, paucity in the applicability of PRF to enhance peri-implant healing in the oral region is not well-corroborated. To evaluate and compare crestal/marginal bone loss around implants placed with Platelet Rich Fibrin (PRF) or without it radiographically.

Materials and Methods: Present study was conducted in the Department of Prosthodontics, Sudha Rustagi College of Dental Sciences and Research, Faridabad. Partially edentulous patients, with at least two teeth missing in a single arch, were selected from the patient pool for this study. Bone loss was assessed in 2 implants placed on the same side or opposite side in the same arch of a single patient. Thus, two implants were placed in the same arch in one patient. Follow up radiographs after implant placement and after three months were analyzed for the amount of bone loss.

Results: Results of the present study showed that on the mesial and distal side of implant placement, clinically significant loss of crestal bone was observed postoperatively when implants were placed with PRF or without PRF. Statically, no significant difference was observed in the level of crestal bone loss mesially and distally when the implant was placed with PRF and without PRF.

Conclusion: Within the limitations of the study, it can be concluded that assessing the effect of placement of PRF in vivo would result in better soft tissue as well as hard tissue healing both quantitatively as well as qualitatively.

Keywords: Growth factors, bone regeneration, blood platelet, platelet-rich fibrin, crestal bone loss, marginal bone loss.

INTRODUCTION

Human life expectancy has been increased by developments in medical science. A longer human life span means that more patients will be partially or fully edentulous. Although the conventional

replacement for lost teeth has been partial or full dentures, the need for a fixed, esthetic, and functional restoration makes dental implants a reliable alternative. Today, dental implants are used

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to support fixed prosthesis or removable overdentures.¹The peri-implant bone quantity and quality not only affects the osseointegration phase but also influences the overlying soft tissues architecture. Assessment of marginal bone levels and peri-implant soft tissues has become an integral part of the evaluation of the implant patient and is usually a significant indicator of implant health. For successful implant therapy, preservation of peri-implant bone is one of the most important factors.² Osseointegration of dental implants is important for long-term success. Various growth factors are expressed during different phases of tissue healing and hence could serve as therapeutic agents to enhance both peri-implant soft and hard tissue repair. Platelet concentrates one of these various growth factors, and platelet-rich fibrin (PRF) is one of the recent innovation of various platelet concentrates.^{2,3} Various studies have substantiated the viability of platelet concentrates on enhancement of osseous and associated tissue healing. He et al. (2009)⁴ evaluated the effect of PRP and PRF on the proliferation and differentiation of rat osteoblasts. They demonstrated that PRF released autologous growth factors gradually, resulting in a stronger and more durable effect on proliferation and differentiation of rat osteoblasts than PRP in vitro. Lee et al. (2012)⁵ evaluated the application of platelet-rich fibrin (PRF) into a peri-implant defect and concluded that the graft using PRF promoted greater amount of bone formation, and the bone-to-implant contact ratio was also significantly higher in the graft using PRF alone than in the unfilled control. However, the propensity of peri-implant tissue to recession after surgical trauma has been well recognized. However, the response of peri-implant tissue loaded with PRF following implant placement is not well documented. PRF efficacy in optimizing and preserving the existing osseous structure and gingival architecture across the peri-implant need to be corroborated. Hence, a Prospective, Randomized Controlled Trial (RCT) was planned to evaluate the effect of Platelet Rich Fibrin (PRF) on crestal/marginal bone loss around implants.

MATERIALS AND METHODS

The present study was conducted in the Department of Prosthodontics, Sudha Rustagi College of Dental Sciences and Research, Faridabad. Partially edentulous patients, with at least two teeth

missing in a single arch, were selected from the patient pool for this study. Bone loss was assessed in 2 implants placed on the same side or opposite side in the same arch of a single patient. Thus, two implants were placed in the same arch in one patient. Ethical clearance was obtained prior to the conduct of this study from the ethical committee, and Informed consent was taken from the patients for inclusion in the study. A total of 12 patients with at least two teeth missing in the same arch were selected according to the inclusion criteria. Twenty Four implants were placed, which were divided into two groups, i.e. PRF group and Non-PRF group. (Table 1)

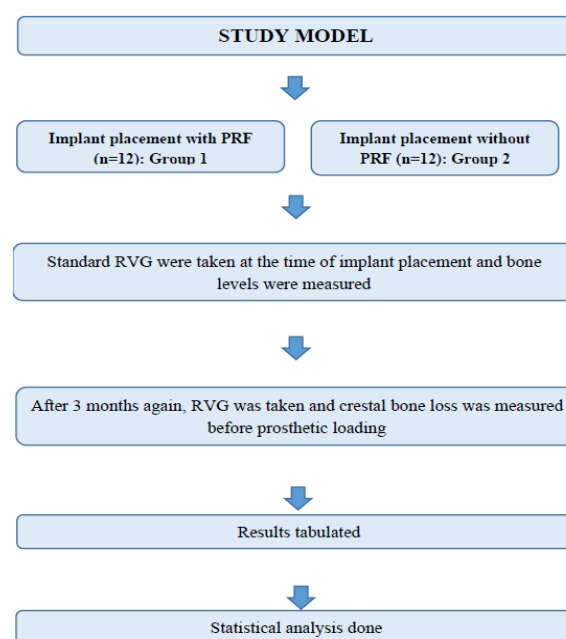


Fig 1: Flow chart representing an overview of the methodology.

PRE SURGICAL PHASE

a) Assessment before surgery

After recording patient history, thorough medical and dental examinations were performed, and pre-operative intra-oral photographs were taken.



Fig 2: Pre-operative intraoral photograph A) maxillary arch B) mandibular arch.

B. Radiographic assessment

Pre-operative RVG images were obtained using Vatech RVG and EZ-dent i software. Panoramic images were obtained using Kodak- digital panoramic machine.



Fig 3: Preoperative A) IOPA B)OPG

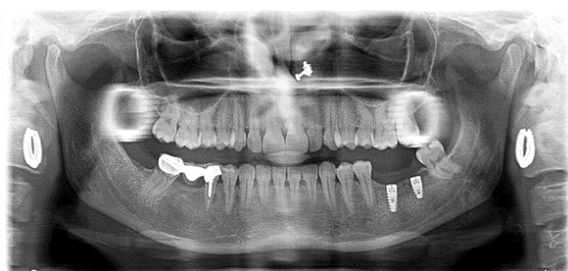


Fig 3c: Immediate post-operative OPG X-Ray.

PLATELET RICH FIBRIN PREPARATION

For the PRF preparation, 10 ml of blood was drawn from the antecubital vein with the help of a syringe and transferred to the test-tube without anticoagulant. The blood sample was immediately centrifuged with the help of centrifugal machine at 3000 rpm for 10-12 minutes, and following layers were observed: red lower fraction containing red blood cells, upper straw-coloured cellular plasma and the middle fraction containing the fibrin clot [Figure 3(a)]. The upper straw-coloured layer was then discarded, and the middle fraction was collected after that fibrin clot was squeezed between gauze-piece to obtain PRF membrane.

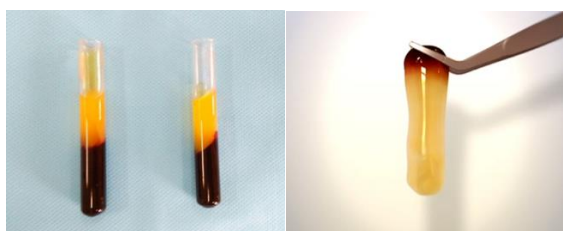


Fig 4: a) Blood in the test tubes after centrifugation at 3000 rpm for 10 minutes divided into three fractions; a lower fraction of red blood cells, a

middle fraction containing fibrin clot and upper acellular plasma fraction b) Isolated PRF.

SURGICAL PHASE

Before the surgical procedure preoperatively X-rays of the surgical site were taken by using standardized paralleling cone technique with the help of a paralleling grid device for treatment planning of implant placement. Standard sterilization protocols were followed for the implant placement. Local anaesthesia was infiltrated around the implant site, and the incision was given along the residual ridge crest, and the mucoperiosteal flap was elevated to expose the ridge crest. Proposed implant sites were marked on the ridge crest with the help of a round bur. The drilling sequence was followed according to the standard surgical protocol, and implants were placed in the osteotomy site flushing it with the margin of the crest. The immediately prepared PRF was then placed over the implant in the test group, and primary closure was done by using 3-0 silk suture. Instructions regarding hygiene maintenance and soft diet plan were given to the patient. After 3-4 months of implant placement in mandible and 4-6 months of implant placement in the maxilla, second-stage surgery was done, and the definitive prosthesis was cemented following standard prosthodontic protocol.



Fig 5: a) Implant placement b) PRF placement.

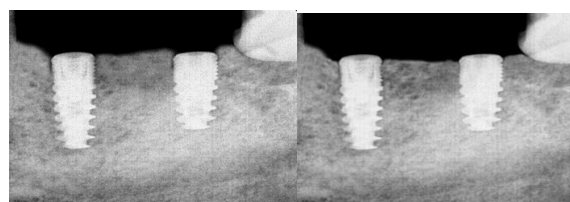


Fig 6: a) Immediate Post operative intra-oral periapical radiograph (b): 3-months post-operative intra-oral periapical radiograph.

Digitizer software was used to record changes in the level of crestal bone. In the software, the most coronal surface of the implant was taken as a static

reference line. The point of bone to implant contact was chosen as the initial bone level. Crestal bone level changes (in mm) were recorded at the mesial and distal aspect of implants by drawing perpendicular from the static reference line to the bone level. Values obtained were tabulated and were subjected to appropriate statistical analysis.⁶

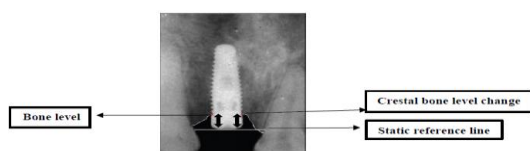


Fig 7: Digimizer software.

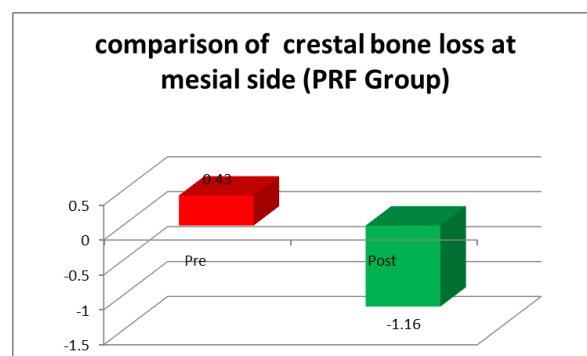
RESULTS

This present study was conducted in Department of Prosthodontics, Crown and Bridge, Sudha Rustagi College of Dental Sciences and Research, Faridabad to evaluate the level of crestal bone loss around implants placed with Platelet Rich Fibrin (PRF) and without Platelet Rich Fibrin (PRF). The implants over which PRF was placed comprised of Group 1 (Study group/ PRF group, n=12) and implants over which no PRF was placed comprised of Group 2 (Control group/Non-PRF group, n=12) The results of this study were obtained by evaluating the data using paired t-test. Crestal bone level changes were observed both in the PRF group and Non-PRF group.

Assessment of crestal bone level at the mesial side (PRF group): In PRF group, crestal bone loss was evaluated at the mesial side immediately after the implant placement (immediate post-operative) and after 3 months of implant placement (3 months post-operative). Mean bone loss value at the mesial side was noted and found to be -0.73 ± 0.83 mm with a standard deviation of 0.260 and 0.77866 mm. The paired t-test was applied, and the result came out to be significant as p-value was less than 0.05.

Table 1: Immediate post-operative and 3-months post-operative comparison of crestal bone levels at the mesial side (PRF Group).

		Mean	N	Std. Deviation	Std. Error Mean	Bone loss Mean $\pm$ SD
	Pre	.43	12	.260	.075	- 0.73 $\pm$ 0.83
	Post	- 1.1600	12	.77866	.22478	
P value	0.011 S					

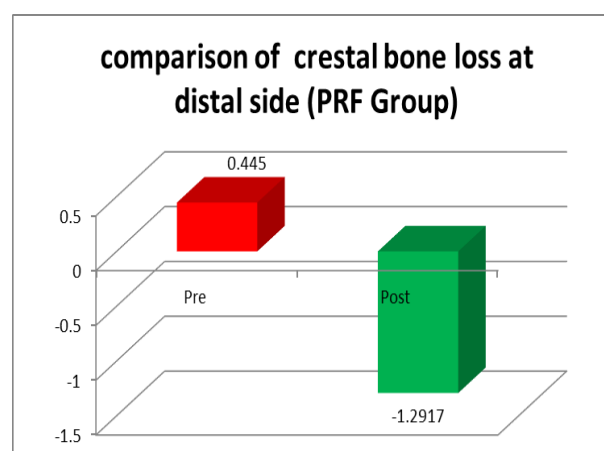


Graph 1: Comparison of crestal bone level at mesial side (PRF Group).

2)Assessment of crestal bone level at distal side (PRF group): In PRF group, crestal bone loss was evaluated at the distal side immediately after the implant placement (immediate post-operative) and after 3 months of implant placement (3 months post-operative). Mean bone loss value at distal side was noted and found to be -0.84 ± 0.64 with standard deviation 0.27487 and 0.68093 mm. Paired t test was applied and result came out to be significant as p-value was less than 0.05.

Table 2: Immediate post-operative and 3-months post-operative comparison of crestal bone levels at the distal side (PRF Group).

		Mean	N	Std. Deviation	Std. Error Mean	Bone loss Mean $\pm$ SD
	Pre	.4450	12	.27487	.07935	- 0.84 $\pm$ 0.64
	Post	- 1.2917	12	.68093	.19657	
P 0.011 S						value



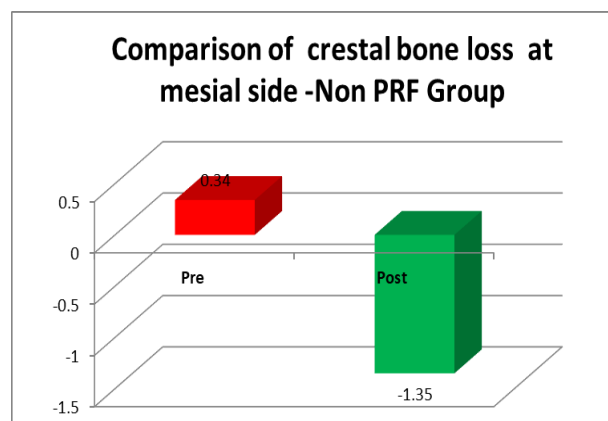
Graph 2: Comparison of crestal bone level at the distal side (PRF Group).

3)Assessment of crestal bone level at mesial side

(Non PRF group): In Non PRF group, crestal bone loss was evaluated at the mesial side immediately after the implant placement (immediate post-operative) and after 3 months of implant placement (3 months post-operative). Mean bone loss value at mesial side was noted and found to be -1.01 ± 0.66 with standard deviation 0.227 and 0.65083 mm. Paired t test was applied and result came out to be significant as p-value was less than 0.05.

Table 3: Immediate post-operative and 3-months post-operative comparison of crestal bone levels at mesial side (Non-PRF Group)

		Mean	N	Std. Deviation	Std. Error Mean	Bone loss Mean $\pm$ SD
	Pre	.34	12	.227	.065	-
	Post	-1.3500	12	.65083	.18788	-1.01 ± 0.66
P value						<0.0001 S



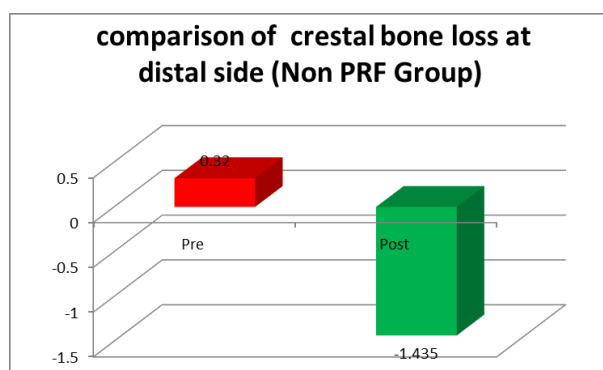
Graph 3: Comparison of crestal bone level at mesial side (Non PRF Group).

4)Assessment of crestal bone level at distal side

(Non PRF group): In Non PRF group, crestal bone loss was evaluated at the distal side immediately after the implant placement (immediate post-operative) and after 3 months of implant placement (3 months post-operative). Mean bone loss value at distal side was noted and found to be -1.11 ± 0.87 with standard deviation 0.24670 and 0.75948 mm. Paired t test was applied and result came out to be significant as p-value was less than 0.05.

Table 4: Immediate post-operative and 3-months post-operative comparison of crestal bone levels at distal side (Non PRF Group).

		Mean	N	Std. Deviation	Std. Error Mean	Bone loss Mean $\pm$ SD
	Pre	.3208	12	.24670	.07122	-
	Post	-1.4350	12	.75948	.21924	-1.11 ± 0.87
P value						<0.0001 S



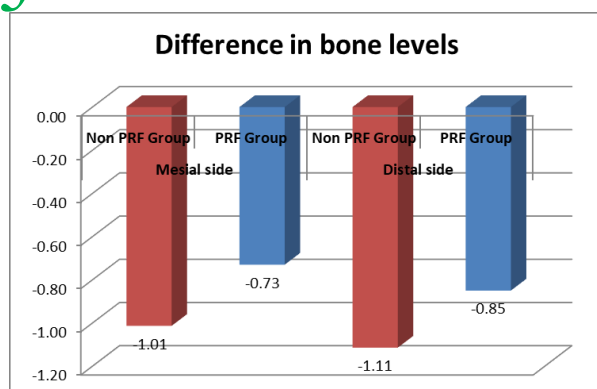
Graph 4: Comparison of crestal bone level at distal side (Non PRF Group).

5) Comparison of crestal bone level between

PRF group and Non PRF group: After evaluation of crestal bone loss in both groups (PRF and Non PRF), values were compared between PRF and Non PRF group. Paired t test was applied and result came out to be insignificant as p-value was more than 0.05.

Table 5: Estimation of difference in bone levels between two groups.

Bone loss		N	Mean	Std. Deviation	Std. Error Mean	P value
Mesial side	Non PRF Group	12	-1.0133	.66220	.19116	0.371 NS
	PRF Group	12	-.7325	.83302	.24047	
Distal side	Non PRF Group	12	-1.1142	.87042	.25127	0.402 NS
	PRF Group	12	-.8467	.64496	.18618	



Graph 5: Difference in bone levels between PRF Group and Non PRF Group.

Table 6: Master chart.

s. no.	group	Pre mesial	Pre distal	Post mesial	Post distal
1	1	0.68	1.13	2.5	2.5
2	1	+0.26	+0.34	1.15	1.4
3	2	0.54	0.44	1.66	1.31
4	2	+0.22	0	0.44	0.74
5	3	0	+0.5	0.77	1.0
6	3	0.42	0.4	1.82	2.26
7	4	+0.18	+0.2	1.12	1.47
8	4	0.37	0.21	1.71	2.1
9	5	0.3	0.6	1.46	1.71
10	5	0	+0.45	0.36	0
11	6	+0.3	0	0.61	1.01
12	6	0.7	0.45	1.76	1.52
13	7	+0.9	+0.5	0.5	0.6
14	7	0	0	1.86	2.1
15	8	+0.29	+0.37	0.4	1.2
16	8	0.37	0.20	2.0	1.9
17	9	0.4	0.4	2.1	2.0
18	9	+0.3	+0.2	1.2	0.9
19	10	+0.4	+0.3	0.8	0.8
20	10	0.5	0.5	2.0	2.2
21	11	+0.8	+0.6	0	0
22	11	0.2	0.2	1.6	1.7
23	12	0.35	0.3	2.0	1.9
24	12	+0.7	+0.9	0.3	0.4

DISCUSSION

The peri-implant bone quantity and quality not only affects the osseointegration phase but also influences the overlying soft tissues architecture. Assessment of marginal bone levels has become an integral part of the evaluation of the implant patient and is usually a significant indicator of implant health. For successful implant therapy, preservation of peri-implant bone is one of the most important factor and in the process of tissue repair and regeneration the quality and quantity of peri-

implant bone can be improved and by harnessing the regenerative capacity of surrounding tissue with the appropriate stimuli. Various growth factors are expressed during different phases of tissue healing and hence could serve as therapeutic agents to enhance both peri-implant soft and hard tissue repair. Platelet concentrates one of these various growth factors, and PRF is one of the recent innovation of various platelet concentrates.^{2,3}

Various studies have been conducted on PRF and its clinical application in various disciplines of dentistry. PRF is used for continuity defects, sinus lift augmentation, horizontal and vertical ridge augmentations, ridge preservation grafting, periodontal defects, cyst enucleation, healing of extraction wounds, endodontic surgeries and to treat gingival recession^{7,8}. All these studies showed that PRF is a healing biomaterial for both soft and hard tissue because of the presence of various growth factors. PRF has been studied mainly for the purpose of bone augmentation and soft tissue healing at other sites. The amount of literature present regarding the soft tissue healing potential of PRF is immense, but at the same time, limited information is available about the hard tissue healing capacity of PRF. Hence, this study was conducted to evaluate the effect of PRF on peri-implant hard tissue to see the changes in the level of crestal bone.

In this study intra-oral, periapical radiographs were used for the assessment of bone level changes. Radiographs were obtained by using the paralleling technique to minimize distortion and standardized by occlusal putty jig. Based upon available bone width and length, implants were placed in PRF sites and Non-PRF sites following standard surgical protocol. PRF was made from patients drawn venous blood using a centrifugal machine (Oxford, India) following manufacturer instruction in terms of time and rpm. This study showed results of high clinical applicability in that it provides information on the effectiveness of PRF on crestal bone changes.

In PRF Group statistically significant crestal bone level changes were noted from baseline to three months, with a mean bone loss change 0.73 ± 0.83 mm mesially with standard deviation 0.260 and 0.77866 mm and mean bone loss changes 0.84 ± 0.64 mm distally with standard deviation 0.27487 and 0.68093 mm. In Non-PRF Group Statistically

significant crestal bone level changes were noted from baseline to three months, with a mean bone loss change 1.01 ± 0.66 mm mesially with standard deviation 0.227 and 0.65083 mm and mean bone loss changes 1.11 ± 0.87 distally with standard deviation 0.24670 and 0.75948 mm. However, the amount of crestal bone level changes as exhibited in the PRF group had a statistically lesser mean value than Non-PRF group.

In this present study, the low mean marginal bone loss was observed in the PRF group. This may be due to Platelet Rich Fibrin (PRF)⁹ which contains high levels of growth factors like PDGF (platelet-derived growth factors), transforming growth factors $\beta 1$ and $\beta 2$ (TGF $\beta 1$, $\beta 2$), vascular endothelial growth factors (VEGF), platelet-derived endothelial growth factors, Interleukin 1&2, basic fibroblast growth factor (β -FGF), platelet-activating factor 4 (PAF-4). These growth factors involved in a cascade of reaction, which involves the immediate binding of secreted growth factors to the transmembrane receptors present on the external surface of cell membranes in graft, flap or wound. This results in the activation of an endogenous internal signal protein, which further initiates the expression of a normal gene sequence of the cell such as matrix formation, cellular proliferation, osteoid production, and collagen synthesis.

The result of this present study is in accordance with the study done by Chang et al. in 2010 who conducted an in-vitro study to evaluate platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts and concluded that PRF could stimulate osteoblasts proliferation. The activation of p-ERK and OPG expression by PRF suggests a potential role for new bone formation. The application of PRF may provide benefit for bone regeneration.¹⁰

The result of this present study is statically insignificant but clinically significant because the low mean marginal bone loss was observed in PRF group because the growth factors are present in PRF as compared to Non-PRF group where there was high mean marginal bone loss was observed. The value of the mean marginal bone loss was 0.3 - 0.4mm lesser in the PRF group as compared to Non-PRF group, which is clinically significant.^{11,12}

Limitations of the study

1. Short follow up time period
2. Small sample size
3. No specified region of maxilla or mandible (or specified quadrant or sextant was not determined).
4. Effect of crestal or subcrustal level implant placement can be further assessed.

CONCLUSION

Positive effects of the PRF was evident in the PRF group. . Radiographically, crestal bone loss was noticeable in PRF sites and Non-PRF sites, but the result was statistically not significant. Although PRF belongs to a new generation of platelet concentrates, the biologic activity of fibrin molecule is enough in itself to account for the significant cicatricial capacity of the PRF. The slow polymerization mode confers to PRF membrane as a particularly favourable physiologic architecture to support the healing process. Within the limitations of the study, it can be concluded that assessing the effect of placement of PRF in vivo would result in better soft tissue as well as hard tissue healing both quantitatively as well as qualitatively.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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