

Single Flap Approach With And Without Platelet Rich Fibrin In The Management Of Intrabony Periodontal Defects – A Randomized Controlled Trial

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Abstract

Aims

This Randomized controlled study was conducted to evaluate the effect of the Single Flap Approach with and without Platelet Rich Fibrin on clinical parameters of periodontal disease, when used to manage intra-bony periodontal defects.

Materials and Methods

Forty intra-bony sites were studied. Twenty sites were surgically accessed with Single Flap Approach + Platelet Rich Fibrin and twenty sites were accessed with Single Flap Approach alone. The Single Flap Approach consisted of an oblique or horizontal butt joint incision is made at the level of the inter-dental papilla. Only the buccal flap was raised while the inter-dental papilla was left in situ. The granulation tissue filling the defect was dissected and removed, leaving the inter-dental and palatal/lingual tissues untouched in both the groups. Additionally, Platelet Rich Fibrin was placed into the defect in Single Flap Approach + Platelet Rich Fibrin group. Primary closure of the flaps was attained with vertical internal mattress suture.

Results

Early wound healing was attained and maintained in all sites in Single Flap Approach + Platelet Rich Fibrin group, while the Probing depth reduction was 3.7 ± 0.17 in Single Flap Approach + Platelet Rich Fibrin group and 3.75 ± 0.91 in Single Flap Approach alone group. The 6-month Clinical attachment level gain was 3.4 ± 0.87 in Single Flap Approach + Platelet Rich Fibrin group and 2.7 ± 1.36 in Single Flap Approach alone group.

Conclusion

SFA+PRF resulted in better early wound healing and greater Clinical Attachment gain than SFA alone.

INTRODUCTION

Periodontal regeneration is contingent on the adsorption, uninterrupted adhesion, and maturation of the fibrin clot positioned between the gingival flap and a periodontal compromised root, that serves as a matrix for the interaction of growth factors, with cells that form the periodontium,^[1] Clot adhesion is dependent on the formation of a resilient union between the fibrin clot and root surface elements. Blood elements placed onto the root surface during surgery or wound closure must establish an attachment to endure normal physiological or other rupturing forces acting on the tooth gingival flap interface and this attachment must remain until such time to offset the impact of functional and other forces,^[2] As our understanding of the events that occur during periodontal healing increases, we have the potential to mimic these temporo- spatial events, which modulate the healing periodontal wound, thereby increasing the probability of periodontal regeneration. During the early phases of healing following the elevation of a gingival flap,^[3]

Flap manipulation should ensure the stabilization of the root surface-adhering blood clot in a biologic environment protected from mechanical and microbiologic challenges,^[4] A dehiscence of the wound margins may occur as a result of a compromised vascular supply due to surgical manipulation and/or tensile forces acting on wound margins,^[5] Wound dehiscence may compromise wound stability, which in turn would jeopardize the cascade of biologic events leading to periodontal regeneration,^[6] The first postoperative weeks seem to be particularly critical for the maintenance of wound stability. Surgical management of the supra-crestal soft tissues, including flap design and suturing technique, seems of paramount importance in controlling the chances of wound failure during the early phases of healing, thus preserving clot stability,^[7] Over the years, new surgical techniques specifically designed to optimize functional and aesthetic features. Cortellini et al. in 1995,^[8] modified the papilla preservation technique that had been proposed earlier by Takei, with the primary purpose of increasing the possibility of primary intention healing during regenerative periodontal surgery. Later, these authors, proposed the simplified papilla preservation flap was for areas with narrower inter-proximal spaces. Utilizing the principles of minimally invasive surgery, they further modified the papilla preservation technique in 2007,^[9] while advocating the Minimally Invasive Surgical Technique (MIST). The MIST was again modified by Cortellini, into the Modified Minimally Invasive Surgical Technique. These modifications to the conventional flap technique ensured that the supra-crestal tissues remain relatively undisturbed even while providing surgical access to the deeper periodontal tissues,^[10,11,12] Biochemical analysis of the PRF composition has indicated that this biomaterial consists of an intimate assembly of cytokines, structural glycoprotein and mesenchymal stem cells that are enmeshed into a three dimensional, slowly polymerizing fibrin network. The biologic activity of the fibrin molecule and the slow polymerization mode confers to the PRF matrix a particularly favorable physiologic architecture to support the healing process,^[13] Recently, studies have demonstrated that the PRF membrane has a very significant slow sustained release of key growth factors for at least one week and up to 28 days, which means that the PRF matrix stimulates its environment for a significant time during wound healing,^[14] Recent studies have evaluated the effects of PRF when used along with open flap debridement. These studies have found that the additive use of PRF, increases Probing Pocket depth reduction and Clinical Attachment gain,^[15] Though the PRF matrix has many favorable features, it has not been considered as an adjunct to any of the Minimally Invasive Periodontal Surgeries. The Single Flap Approach and the Platelet Rich Fibrin Matrix, both have the potential to positively alter periodontal wound healing,^[16] The current study hypothesized that the additive effect of the Single Flap Approach and the use of Platelet Rich Fibrin Matrix as a graft could further favorably alter periodontal healing following periodontal surgery, as evaluated by Probing Pocket depth reduction and Clinical Attachment gain. This study therefore compared the effect of Single Flap Approach to the combined effect of

Single Flap Approach with additional use of a Platelet Rich Fibrin Matrix, on the clinical parameters of periodontal disease.

MATERIALS AND METHODS

A randomized controlled clinical trial was conducted among subjects aged 25 -50 years in Chennai between 2018 and 2020. The purpose of this study was to test if the additive effect of platelet rich fibrin matrix on the healing periodontal wound following treatment of isolated intra-bony defect with Single Flap Approach will favorably alter the clinical outcomes on comparison with Single Flap Approach alone. This study was approved by the Institutional Ethical Committee of the SRM University. Patients were explained about the study and a written informed consent was obtained from the patients willing to participate in the study. Using G power, sample size was calculated based on the proportion set at 0.15 with Type II error β set at 90% and type I error α set at 5%. The estimated sample size was 12 sites in each group. Taking the possibility of dropouts into consideration, 20 sites were recruited in each group with Type II error β set at 90%.

SELECTION CRITERIA

Inclusion Criteria -

- Age- 25-50 years,
- > 5 mm of probing pocket depth, that was isolated and not circumscribing the tooth.
- > 3mm Clinical attachment loss, that was isolated to the region of pocket formation, and not circumscribing the tooth.
- Angular bone loss that was either a two or three walled defect, as evaluated by trans-gingival probing.
- No systemic diseases that contraindicated periodontal surgery.
- Presence of Full Mouth Plaque Score \leq 20%.
- Presence of Full Mouth Bleeding Score \leq 25%.
- Normal Platelet count ($>200,000$ cells/ mm^3).

EXCLUSION CRITERIA

- Pan chewers, smokers,
- Pregnant patients,
- Patients with malocclusion,
- Teeth with Class II or Class III furcation involvement,
- Teeth with grade III mobility

Following administration of 2% Local anesthesia with 1:80000 adrenaline, Buccal infiltration with inferior alveolar nerve block/greater palatine nerve block was given depending on which quadrant is the defect site. Sulcular incisions are made following

the gingival margin of the teeth included in the surgical area. An oblique or horizontal butt joint incision is made at the level of the interdental papilla. The elevation of a buccal mucoperiosteal flap allows for proper root/defect debridement done without touching the interdental papilla. Intra surgical measurements -The depth, width and number of walls of the defect were determined during the surgery using UNC 15 Probe. Preparation of PRF - A 10 ml blood sample was taken from the patient in 10 -ml tube which is immediately centrifuged in a table centrifuge at 2,500 rpm (approximately 400 g) for 10 min. The PRF was prepared in accordance with the protocol developed by Choukroun et al. Just prior to surgery, intravenous blood (from the ante-cubital vein) was collected in a 10-ml sterile glass tube without anticoagulant and immediately centrifuged in centrifugation machine at 3000 rpm for 10 minutes. It resulted in separation of blood into a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (Platelet-poor plasma) at the top. PPP was discarded, PRF was easily separated from red corpuscles base using sterile tweezers and scissors. The junction of PRF to RBC layer was preserved as this region is supposed to be richest in all the growth factors,[17] Transferring and immobilizing the graft - The PRF was adapted into the defect site. Wound closure is obtained with 5 -0 vertical internal mattress suture. Periodontal dressing - Periodontal dressing (COE – PAK) was placed. Medications Prescribed – Patients were prescribed with analgesic medication (Ibuprofen 400mg every 8 hours) for three days. Oral hygiene maintenance recommended - Chlorhexidine [0.12%] was recommended twice a day for 2 weeks. Patients were instructed not to brush the area for 2 weeks post-surgically in order to avoid damage to the gingival collar. Periodontal dressing and Suture removal - The dressing was removed after two weeks and suture removal done in relation to the defect associated site with proper saline irrigation.

SINGLE FLAP APPROACH

Following administration of 2% LA with 1:80000 adrenaline, Buccal infiltration with inferior alveolar nerve block /greater palatine nerve block was given depending on which quadrant is the defect site.

- Sulcular incisions are made following the gingival margin of the teeth included in the surgical area.
- An oblique or horizontal butt joint incision is made at the level of the interdental papilla.
- The elevation of a buccal mucoperiosteal flap allows for proper root/defect debridement done without touching the interdental papilla. Periodontal dressing - Periodontal dressing (COE – PAK) was placed.
- Medications Prescribed - Patients were prescribed with analgesic medication (Ibuprofen 400mg every 8 hours) for three days)
- Oral hygiene maintenance recommended - Chlorhexidine [0.12%] was recommended twice a day for 2 weeks. Patients were instructed not to brush the area for 2 weeks post-surgically in order to avoid damage to the gingival collar.
- Periodontal dressing and Suture removal - The dressing was removed after two weeks and suture removal done in relation to the defect associated site with proper saline irrigation. All patients were called for reviews at second week, third month and sixth month.

- Patient recalled at second week for removal of periodontal dressing and suture. Early wound healing index,^[18] was evaluated.
- Recalled at 3rd and 6th month for evaluation of clinical parameters such as, Full mouth Plaque score, ^[15] Full mouth bleeding index, ^[15] Probing pocket depth (PPD), Clinical attachment level (CAL).

All patients maintained adequate plaque control during the course of therapy. Professional maintenance care was performed at each recall visit. Statistical analysis was done using SPSS software version 25.0, the descriptive statistics was done to assess the index scores recorded and inferential statistics was done using independent sample t test.

RESULTS

Table 1 shows the comparison of plaque index score. The increase in plaque levels was statistically highly significant in the baseline to three-month group, on comparison with the levels of plaque, from baseline to six months in the SFA alone group.

Table 1: Intra comparison of in Full mouth plaque scores – Bonferroni Test

FMPS Group	Bonferroni Test	Mean diff	Std. error	Sig.	95% confidence Interval	
					Lower bound	Upper Bound
SFA + PRF						
Comparison of Baseline to 3 month		-7.777	1.055	0.0001*	-10.38	-5.17
Comparison of Baseline to 6 month		-8.589	1.055	0.0001*	-11.19	-5.99
Comparison of 3 month to 6 Month		-0.812	1.055	1.000	-3.42	1.79
SFA alone						
Comparison of Baseline to 3 months		-7.358	1.68	0.0001*	-11.5	-3.21
Comparison of Baseline to 6 months		-5.116	1.68	0.011*	-9.26	-0.97
Comparison of 3 month to 6 Month		2.242	1.68	0.562	-1.9	6.39

Table 2 shows the inter-group comparison of full mouth plaque score. These results indicate that the levels of plaque had increased significantly from baseline to six months, post-surgical management of the defects. This increase was seen in both the test and the control groups. On comparison of the plaque levels between groups, there was no difference at baseline and at three months. However, at six months on comparison between groups there as a statistically

significant difference at the level of $p < 0.03$. The same is represented in the graph 1.

Table 2: Inter Group comparison of Full Mouth Plaque Score – Students t Test

	GROUP	N	Mean	Std. Deviation	Std Error Mean	P-value
Baseline	SFA+PRF	20	18.19	0.7232	0.1617	0.629
	SFA alone	20	17.86	0.9105	0.2036	
Three Months	SFA+PRF	20	25.97	2.521	0.564	0.65
	SFA alone	20	25.22	6.8663	1.535	
Six Months	SFA+PRF	20	26.78	4.381	0.98	0.03*
	SFA alone	20	22.98	6.031	1.349	

Graph 1: Inter group comparison of Full Mouth Plaque Score

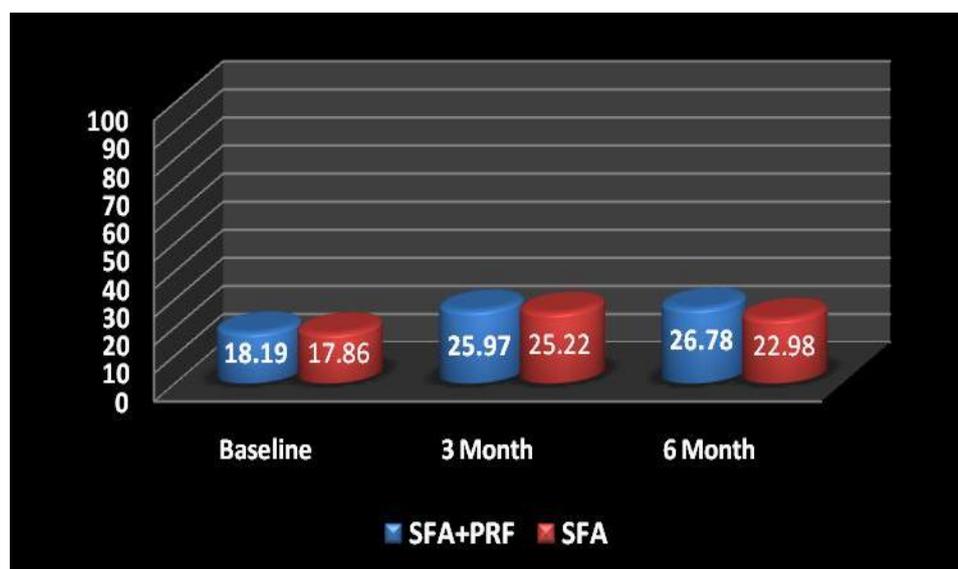


Table 3 shows the intra-group comparison of full mouth gingival index. The gingival bleeding score was calculated at specific time intervals to identify if the gingiva was presenting signs of gingival inflammation. The Full mouth bleeding Scores were calculated to indicate the overall gingival inflammation level in the mouth during the period of investigation. The gingival bleeding score in the SFA alone group had a mean score of 22.61 at baseline, which decreased to 15.89 at three months and thereafter slightly increased to 16.5. In the SFA + PRF group, the baseline score of 22.61, reduced at the three month point to 18.12 before increasing to 28.61 at the six-month point. On comparison of the gingival inflammatory status within the groups, only the SFA alone group had a mildly significant difference at the third month point.

Table 3: Intra comparison of Full mouth bleeding scores- Bonferroni Test

FMBS-	Bonferroni test				95% confidence interval	
Group		Mean diff	Std. error	Sig.	Lower bound	Upper bound
SFA + PRF						
Comparison of Baseline to 3 months		0.232	0.416	0.584	-0.639	1.1034
Comparison of Baseline to 6 months		-10.086	0.804	1	-11.768	-8.4025
Comparison of 3 month to 6 months		-10.31	1.037	0.698	12.488	-8.1467
SFA alone						
Comparison of Baseline to 3 months		6.716	2.528	0.031*	48	12.95
Comparison of Baseline to 6 months		6.11	2.528	0.057	-0.13	12.34
Comparison of 3 month to 6 month		-0.606	2.528	1	-6.84	5.63

Table 4 shows the comparison of the full mouth bleeding score between groups, at baseline there was a statistically mildly significant difference ($p < 0.04$), due to the increased level of gingival bleeding in the SFA alone group. There was no statistically significant difference at three months between the two groups. At six months there was an increase of FMBS score in the SFA+PRF group and this was statistically highly significant ($p < 0.0001$). The same is represented as a graph below.

Table 4: Inter Group statistics of Full mouth bleeding score

	GROUP	N	Mean	Std. Deviation	Std Error Mean	P-value
Baseline	SFA+PRF	20	18.36	1.375	0.307	0.037
	SFA alone	20	22.61	8.678	1.94	
Three months	SFA+PRF	20	18.12	1.497	0.334	0.133
	SFA alone	20	15.89	6.327	1.415	
Six months	SFA+PRF	20	28.45	4.414	0.987	0.0001
	SFA alone	20	16.5	8.738	1.954	

Graph 2: Inter group comparison of Full Mouth Bleeding Score

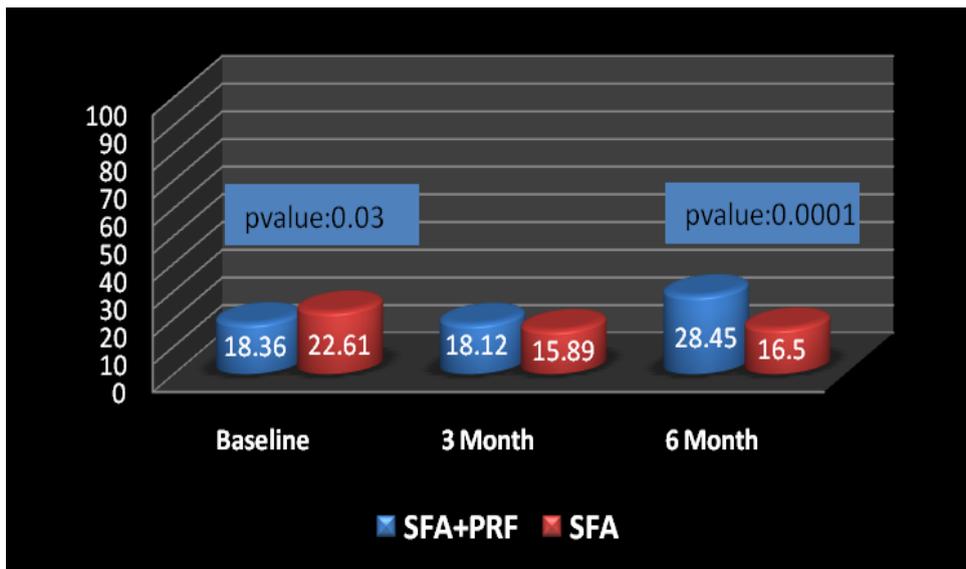


Table 5 shows the intra-group comparison of probing pocket depth. The decrease in probing pocket depth, when compared from baseline to three months and six months for the test and control group was highly significant ($p < 0.0001$). In the SFA alone group, the comparison of the decrease in probing pocket depth from baseline to three months and from baseline to six months was only mildly significant at the level of $p < 0.02$.

Table 5: Intra-Group Comparison of Probing pocket depth reduction-t-Test for Probing pocket depth reduction

Probing Pocket Depth	Bonferroni Group	Mean Difference	Std. error	Significance	95% confidence interval	
					Lower bound	Upper bound
SFA + PRF						
Comparison of Baseline to 3 months		2.175	0.173	0.0001	1.764	2.604
Comparison of Baseline to 6 months		3.70	0.173	0.0001	3.271	4.129
Comparison of 3 month to 6 months		1.52	0.173	0.0001	1.096	1.954
SFA						
Comparison of Baseline to 3 months		3.175	0.252	0.0001	2.55	3.8
Comparison of Baseline to 6 months		3.625	0.252	0.0001	3	4.25
Comparison of 3 month to 6 Months		0.45	0.252	0.239	-0.17	1.07

Table 6 shows the Intergroup clinical Attachment Level gain was significant on comparison of the gain from baseline to three months ($p < 0.02$). However, there was no significant difference on comparing the baseline to six months or when comparison with the three months to six-month score.

Table 6: Intra comparison of Clinical attachment level gain- Bonferroni Test

CAL	Bonferroni test				95% confidence Interval	
Group		Mean diff	Std. error	Sig.	Lower bound	Upper Bound
SFA + PRF						
Comparison of Baseline to 3 months		2.9	0.200	0.0001	2.48	3.47
Comparison of Baseline to 6 months		3.4	0.200	0.0001	2.93	3.92
Comparison of 3 month to 6 months		0.4	0.200	0.86	-0.04	0.94
SFA alone						
Comparison of Baseline to 3 months		2.1	0.268	0.0001	1.44	2.76
Comparison of Baseline to 6 months		2.7	0.268	0.0001	2.04	3.36
Comparison of 3 month to 6 months		0.6	0.268	0.087	-0.04	0.94

Discussion

The platelet concentrate enmeshed in a fibrin network, is steeped in growth factors such as Platelet Derived Growth Factor-AB, Transforming Growth factor and Vascular Endothelial Growth factor, that are released in sustained manner for over one week and upto twenty-eight days. Recent studies have also shown that PRF is capable of stimulating the proliferation of periodontal ligament fibroblasts, osteoblasts and also increase the secretion of Osteoprotegerin, which is probably due to its capability to up-regulate the signaling molecule, Extracellular sign - Regulated Protein kinase (ERK),^[17] This study was performed to assess the efficacy of the Single Flap Approach with and without Platelet Rich Fibrin, on clinical parameters of periodontal disease to evaluate the potential additive effects of PRF when used with the Single Flap Approach. To this end forty intra-bony defects in systemically healthy patients, were randomly assigned to receive treatment by the Single Flap Approach with platelet Rich Fibrin or the Single Flap Approach alone. The clinical parameters evaluated were the Full Mouth Plaque Score, the Full Mouth Bleeding Score, the Early Wound Healing Index,^[18] the Probing Pocket depth reduction, and the Clinical Attachment level Gain.

The early wound index is scored from one to five, indicating complete closure for the score of one and incomplete flap closure with complete necrosis of the inter-proximal tissue for the score of five. In the SFA+PRF group all the twenty sites healed with a score of one

indicating complete flap closure with no fibrin line at the site of inter proximal incision, when the patient was examined after two weeks, for suture removal. However, in the control SFA alone, two sites scored two on the Early Wound Healing Index (healing with a thin fibrin line inter-proximally), while all other sites scored one on the index. This difference between the two groups was however statistically not significant. This indicates that all the forty sites that underwent surgical intervention, showed a high degree of wound margin stability, which was probably reflective of the entire wound stability,^[18] The next parameter evaluated was the level of dental plaque that was present as assessed through the dichotomous O'Leary index,^[15] which was used to calculate the Full Mouth Plaque Score. Measurements were made at baseline, at three months and at six months. As per the inclusion criteria the Full mouth Plaque scores were below 20 percent in both the groups (18.19 for test and 17.86 for control). However, when the patients were evaluated at three months and at six months the FMPS scores had become higher, it was 25.97 and 25.22 as mean for the test group, at the two post-surgical time points. In the control group the FMPS had also increased to a mean of 25.22 and 22.98. This difference within the two groups on comparison from baseline to three and six months was highly significant. There was also a mild significant difference on comparison of the FMPS scores between the two groups at the six-month time point ($p < 0.03$). In literal terms this meant that about seven to eight teeth, (out of thirty-two teeth) in the oral cavity scored for the presence of plaque in the dichotomous Full Mouth Plaque Score, at the highest score.

The next parameter evaluated was the Full Mouth Bleeding Score, which is an indicator of the number of teeth around which the gingiva is inflamed. The gingival bleeding in the SFA+PRF group had increased from baseline and three months to six months. In the control SFA group the gingival bleeding score had decreased from baseline to the three- and six-month time point. The difference from baseline to three months in the control group was statistically significant at $p < 0.03$. At the six month point in the SFA+PRF group, four sites presented with gingival inflammation. On comparison between groups the baseline scores were significantly higher in the SFA alone group. However, at the six month time point, the difference between SFA and SFA+PRF was highly significant at $p < 0.0001$. When the Ainamo and Bay index^[16] was used to identify the number or sites that presented with gingival bleeding, only three sites were positive for gingival inflammation in the SFA alone group, as well as at the three month point in the SFA+PRF group. At the six month point in the SFA+PRF group four sites were positive for gingival inflammation. This indicates that despite the slightly higher Full Mouth Bleeding Score, at the review points, most of the sites that underwent surgical intervention did not exhibit signs of inflammation.

Probing pocket depth reduction is one of the primary objectives of the Single Flap Approach. In the SFA+PRF group, the Probing Pocket depth had highly significant decreases, on comparison between all-time points. In the SFA alone group, however, the highly significant comparisons were from baseline to three- and six-month time points. The mean reduction of Probing pocket depth in the SFA+PRF group was 3.70mm at six months while in the SFA alone group 3.75mm. When the intra-group comparisons were evaluated for statistical significance, the SFA+PRF group had a highly significant difference with p values < 0.0001 , for the difference between pocket reduction between the two review time points. In the SFA alone group the reduction of Probing Pocket depth between the two points was significant with $p < 0.02$. This was probably

Inter group evaluation for reduction in probing pocket depth was statistically highly significant when evaluating the difference in probing pocket depth reduction at the three-Month point ($p < 0.0001$). Inter group comparison for pocket depth reduction was not significant at the six-month time point. The probing pocket depth reduction reveals that in the SFA alone group greater probing pocket depth had occurred by the three-month period. However, by the six-month time point both the groups had equal amounts of probing pocket depth reduction.

The clinical attachment gain is also reflected in the probing pocket depth reduction. Nevertheless, the SFA group alone reveals a greater amount of probing pocket depth reduction at that is significant at three months. This increase in probing pocket depth reduction in the SFA alone group, that is not reflected in the Clinical Attachment gain, is probably due to increase in post-surgical gingival recession. This study did not record baseline gingival position and is therefore not able to comment on this aspect of the healing of the periodontal tissues, and is therefore a major limitation. Laurell in 1998^[19], reviewed the effects of Open Flap Debridement, on clinical parameters of periodontal disease. They reviewed the results of twenty-one studies on the effects of various periodontal procedures. They found that Open Flap debridement resulted in limited Clinical Attachment gain, that averaged 1.5mm.

The patients who were treated in this study were asked to maintain appropriate oral hygiene, through the use of both mechanical and chemical plaque control. They were to return during the review appointment to schedule themselves for an oral prophylaxis. Despite these rigors the patients in the SFA+PRF group revealed higher plaque scores and scored higher on the Full mouth Plaque Score and the Full mouth bleeding scores. It is possible that more aggressive supportive treatment to these cases, might further positively affect the treatment groups in this study. This study had other limitations. While this was a randomized controlled trial, examiners could have been blinded to remove any inbuilt bias in those who evaluated the parameters studied. Also, measurements of radiographic bone fill could have been attempted. Despite these limitations, this study has proved in principle that Platelet Rich Fibrin when grafted to a periodontal defect, which is approached through a minimal access periodontal surgical technique, has a high potential to favorably modulate the healing periodontal.

CONCLUSION

This study validates the significant improvements that have been shown in earlier studies that have treated intra-bony defects with minimal access and with primary intention healing in the inter-proximal region. Our study has also further clarified that Platelet Rich Fibrin used as an autologous. Bioactive matrix has a key role to play in attempts to make Periodontal Regenerative Surgery, predictable and cost effective, so that it may become the standard of care for management of periodontal defects. While further studies are required that address the limitations of this study, this study has been proof that minimal access surgical technique, to facilitate primary wound closure when used along with bioactive matrices can enhance the innate healing potential in the periodontium to result in periodontal regeneration.

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