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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

Fortyintra-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 defectin Single Flap Approach + Platelet Rich Fibrin group. Primary closure of the flaps was attained with vertical internal mattresssuture.

Results

Early wound healing was attained and maintained in all sites in Single Flap Approach + PlateletRich

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-monthClinical attachment level gain was 3.4± 0.87in Single Flap Approach +Platelet Rich Fibringroup and 2.7 ± 1.36 in Single Flap Approach alonegroup.

Conclusion

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

Keywords: Wound healing, platelet rich fibrin, single flap approach, clinical attachment

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 2018and 2020. The purpose of this study was to test if theadditive 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 approvedbythe 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 IIerror β set at 90% and type I error α set at 5%. The estimated samplesize 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 at90%.

SELECTION CRITERIA

Inclusion Criteria -

- Age- 25-50years,
- > 5 mm of probing pocket depth, that was isolated and not circumscribing thetooth.
- >3mm Clinical attachment loss, that was isolatedto theregion of pocket formation, and not circumscribing thetooth.
- Angular bone loss that was either a two orthree walled defect, as evaluated by trans-gingivalprobing.
- No systemic diseases that contraindicated periodontal surgery.
- Presence of Full Mouth Plaque Score ≤20%.
- Presence of Full Mouth Bleeding Score ≤25%.
- Normal Platelet count (>200,000cells/ mm^3).

EXCLUSION CRITERIA

- Pan chewers,smokers,

- Pregnantpatients,
- Patients withmalocclusion,
- Teeth with Class II or Class III furcationinvolvement,
- Teeth with grade IIImobility

Following administration of 2% Local anesthesiawith 1:80000adrenaline,Buccal infiltration with inferior alveolar nerveblock/greater palatine nerve block was given depending on which quadrant is the defect site. Sulcular incisionsare made following the gingival margin of the teeth included in the surgicalarea.An oblique or horizontal butt joint incision is made at the level of theinter dentalpapilla. The elevation of a buccal muco-periosteal flap allows for proper root/defect debridement done without touching theinter dentalpapilla.Intra surgical measurements -The depth, width and number of walls of the defect were determined during the surgery using UNC 15Probe.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 10min. The PRF was prepared in accordance with the protocol developed by Choukrounet al. Just prior to surgery, intravenous blood (from the ante-cubital vein) wascollected 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 wasdiscarded, PRF was easily separated from red corpuscles base using sterile tweezers and scissors. The junction of PRF to RBClayer was preserved as this region is supposed to be richest in allthe growth factors,[17] Transferring and immobilizing the graft-The PRF was adapted into the defectsite. Wound closure is obtained with 5 -0 vertical internal mattresssuture. Periodontal dressing - Periodontal dressing (COE – PAK) wasplaced. Medications Prescribed – Patients were prescribedwith analgesic medication Ibuprofen 400mg every 8 hours) for threedays.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 gingivalcollar.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 salineirrigation.

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 surgicalarea.
- An oblique or horizontal butt joint incision is made at the level of the inter dentalpapilla.
- The elevation of a buccal mucoperiosteal flap allows for proper root/defect debridement done without touching the interdentalpapilla. Periodontal

dressing - Periodontal dressing(COE – PAK) wasplaced.

- Medications Prescribed - Patients were prescribed with analgesic medication (Ibuprofen 400mg every 8 hours) for threedays)
- 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 gingivalcollar.
- 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 salineirrigation. All patients were called for reviews at second week, third month and sixthmonth.
- Patient recalled at second week for removal of periodontal dressing and suture. Early wound healing index,[18]wasevaluated.
- Recalled at 3rdand 6thmonth for evaluation of clinical parameters such as, Full mouth Plaque score,[15]Fullmouth bleeding index,[15]Probing pocket depth (PPD), Clinical attachment level(CAL).

All patients maintained adequate plaquecontrol during the course of therapy. Professional maintenance care was performed at eachrecallvisit. 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	**Bonferroni Test**				**95% confidence Interval**	
Group		**Mean diff**	**Std. error**	**Sig.**	**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 asastatistically 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

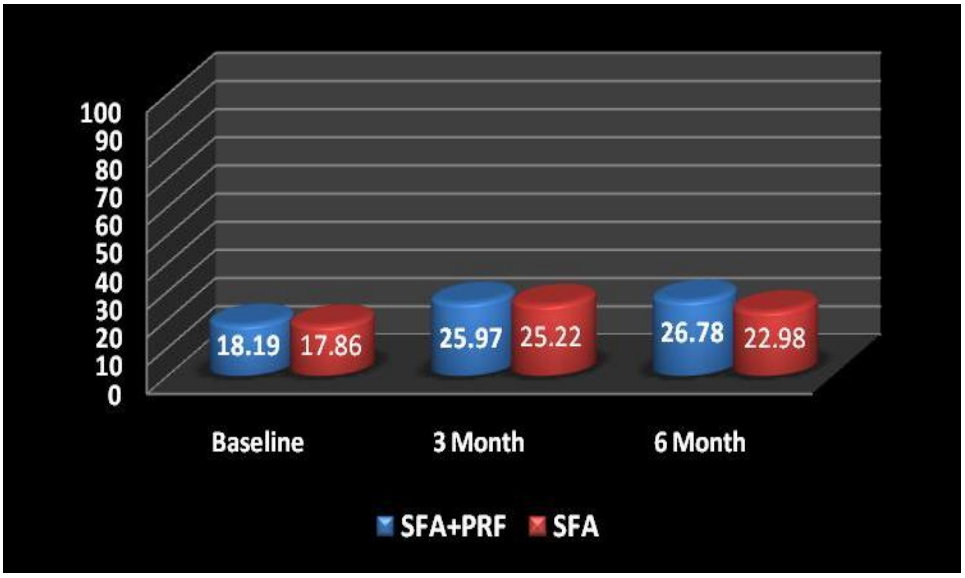

Table 3 shows the intra-group comparison of full mouth gingival index. The gingival bleeding score was calculated at specific time intervals to identify if the gingiva was presenting signs of gingival inflammation. The Full mouth bleeding Scores were calculated to indicate the overall gingival inflammation 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 mildlysignificant 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 scorein 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

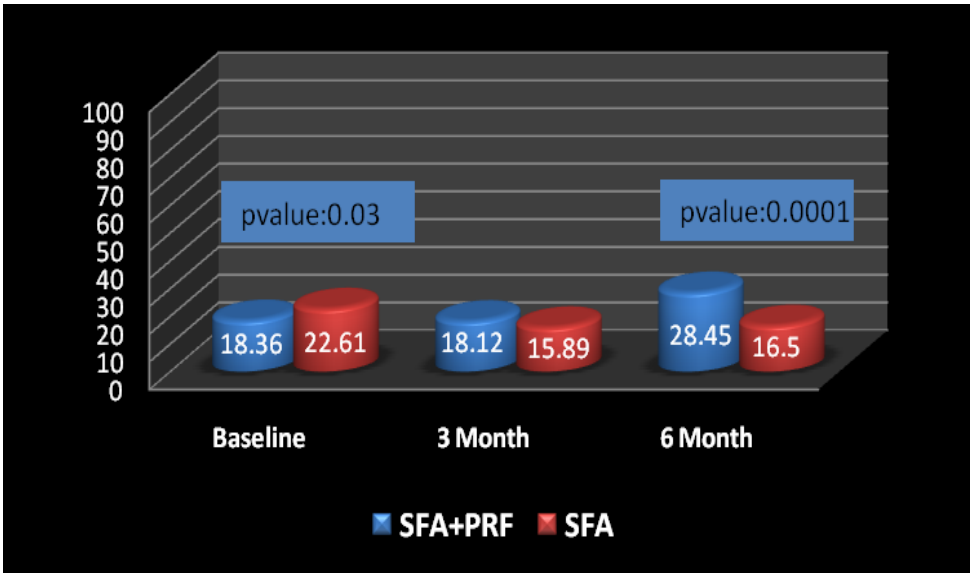

Table 5 shows the intra-group comparison of probing pocket depth. The decrease in probing pocket depth, when compared from baseline to three months and six months for the test and control group was highly significant (p-<0.0001). In the SFA alone group, the comparison of the decrease in probing pocket depth from baseline to three monthsand from baseline to six months wasonly mildly significant atthe 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				95% confidence interval	
	Group	Mean Difference	Std. error	Significance	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-monthscore.

Table 6: Intra comparison of Clinical attachmentlevelgain-BonferroniTest

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 factorand Vascular Endothelial Growth factor, that are released in sustained manner for over one week and uptotwenty-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 Osteo-protegrin, 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 reductionandtheClinical Attachment levelGain.

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 tissuefor 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 woundmargin stability, which was probably reflective of the entire wound stability,[18]Thenext parameter evaluated was the level of dental plaque that was present asassessed throughthe 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 atthreemonths 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 meanof25.22and22.98. 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 highestscore.

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 gingivalbleeding in the SFA+PRF grouphadincreased from baseline and three months to six months. In the control SFA group the gingival bleeding score had decreasedfrom baseline to the three- and six-monthtime point. The difference from baseline to three monthsin thecontrol group was statistically significant at $p<0.03$. At thesixmonth point in the SFA+PRF group, foursites presented with gingival inflammation. On comparison between groups the baseline scores were significantly higher in the SFA alone group. However,at the six monthtime point, the difference between SFA and SFA+PRFwashighly significant at $p<0.0001$. When the Ainamoand Bay index [16]wasusedtoidentifythenumberorsitesthatpresentedwith gingival bleeding, only three sites were positivefor 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 thatunderwent surgical intervention did not exhibit signsof 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-monthtime 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 theintra-group comparisons were evaluated for statistical significance, the SFA+PRF group had a highly significant difference with p values <0.0001, for thedifferencebetweenpocket 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 statisticallyhighlysignificant whenevaluatingthedifference inprobing pocket depth reduction at thethree-Month point ($p<0.0001$). Inter group comparison for pocket

depth reduction was not significant at the six-monthtime point. The probing pocket depth reduction reveals that in the SFA alone group greater probing pocket depth had occurred by the three-monthperiod. However,by the six-monthtime point both the groups had equal amounts of probing pocket depthreduction.

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 notreflected in the Clinical Attachment gain,is probably due to increase in post-surgicalgingival 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 majorlimitation.Laurellin 1998[19], reviewed the effects of Open Flap Debridement, on clinical parameters of periodontal disease. They reviewed the results of twenty-onestudies onthe effects of various periodontal procedures. They found that Open Flap debridementresulted 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 appointmenttoschedule 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 thisstudy. 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 RegenerativeSurgery, 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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CONFLICTS OF INTEREST: Nil

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