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Effect of platelet rich fibrin membrane on gingival wound healing by secondary intention: A clinical trial

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Abstract

Gingival enlargement, a frequent entity that can be managed by scalpel gingivectomy. However, healing occurs by secondary intention. So, to promote healing Platelet rich fibrin (PRF) can be used as it is a rich source of growth factors. The aim of the present study was to evaluate the effect of PRF membrane on pain perception and gingival wound healing by secondary intention. A total of 10 subjects, requiring gingivectomy, were selected. A split mouth designed study was planned. After gingivectomy, in control group - Coe-Pak alone was applied, and in test group - PRF was applied under Coe-Pak. Visual analogue scale (VAS) for pain response on 1st, 3rd, 5th, 7th post-operative days and healing index (Landry and Turnbull, 1988) on 5th, 7th, 15th and 30th post-operative days were applied to assess the response. On intragroup comparison between 3rd and 5th post-operative days, test group showed statistically significant pain reduction (p=0.015) compared to control group (p=0.081) between the same period. The mean scores of healing index were

better in control group than test group on 5th post-operative day (2.80±0.789, 2.70±1.059 respectively), but they were better in test group than control group on 7th (4.10±0.876, 3.60±0.966, respectively) and also on 15th post-operative day (4.90±0.316, 4.80±0.422, respectively). However, intergroup comparison showed statistically insignificant difference at all time intervals for both pain and healing indices (p>0.05). So, It can be concluded that PRF may be safely used as a soft tissue dressing in open wound to achieve uneventful healing. However, future studies with larger sample size should be carried out to establish definitive result.

Keywords: Gingival enlargement, Gingivectomy, Platelet rich fibrin, Platelet concentrate.

Introduction

A major goal of periodontal therapy is to re-establish anatomical and physiological conditions conducive to long-term health and function of periodontium.[1] One of the gingival diseases that mostly disturbs tooth aesthetic and function is the gingival enlargement.[2] Hyperplasia and/or overgrowth of the gingiva is rather common and related to a variety of etiologic factors and pathogenic processes (e.g, dental plaque, mouth breathing, hormonal imbalance, medications).[3] Different gingivectomy methods can be used for the elimination of gingival overgrowth; of which the most common is scalpel.[4] Scalpel has advantages of being easy to be used, precise incision with well-defined margins, the healing is fast, and there is no lateral tissue damage.[4] While the disadvantage of scalpel are bleeding that result in inadequate visibility, post-operative discomfort to the patient and healing by secondary intention.[5] Different platelet concentrates are used as a therapeutic tools to improve tissue repair particularly in periodontal wound healing.[6] One of the platelet concentrate used nowadays is Platelet-rich fibrin (PRF) which was first developed in France for use in the field of oral and maxillofacial surgery by Choukroun et al. (2001).[7] So, to enhance healing PRF can be used as the platelets are the rich source of growth factors such as connective tissue growth factor (CTGF), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-1), platelet factor4 (PF-4), platelet-derived growth factor (PDGF),transforming growth factor(TGF-β, including β-1 and β-2-isomers) and vascular endothelial growth factor (VEGF).[6] On the basis of this background knowledge, additional effect of PRF during wound repair after gingivectomy may be expected resulting into more favorable result. However there are several studies: which have not found any additional beneficial effect of PRF.[8,9] Hence, the present study was designed to evaluate the role of PRF, if any, on gingival wound healing by secondary intention.

Material and methods

A randomized controlled double blinded clinical trial using split mouth designed study was conducted. Ethical clearance was obtained from Kothiwal Dental College & Research Centre with reference no. KDCRC / IERB / 10/2018/25. Subjects were selected based on the selection criteria and informed consent was obtained after explaining the procedure.

A. Inclusion criteria

Subjects requiring excisional gingival procedures undergoing healing by secondary intention, subjects with gingival enlargement grade ≥2 (Bokenkamp and Bohnhorst, 1994)[10] involving at least 3 teeth on each side of the midline in an arch (figure 1), subjects with plaque index ≤1 on the day of surgery. (Turesky et al., 1970).[11]

B. Exclusion criteria: Subjects taking any medication which can influence the gingival response during healing, any systemic disease affecting the periodontium, pregnant women, lactating mothers, postmenopausal women, subjects with any blood dyscrasia or bleeding disorder,

smokers and tobacco chewers, subjects who are unable to perform routine oral hygiene procedures or not complying with oral hygiene instructions.

Finally ten subjects were selected for the study, requiring gingivectomy on contralateral side. From each subject two selected sites were randomly assigned into two treatment groups (Group A & Group B) by drawing chits, stating control group or test group respectively. Group A (Control group):- sites for Periodontal dressing, Coe Pak (GC America INC ALSIP, IL 60803, USA) alone. Group B (Test group):- sites for PRF membrane under Coe Pak.

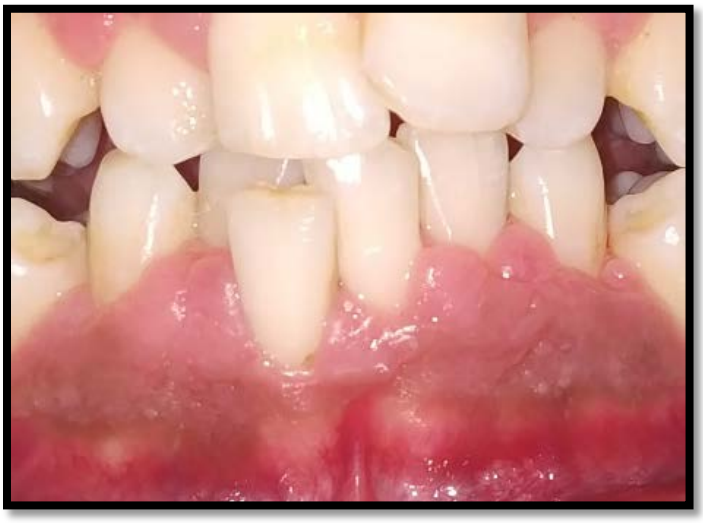
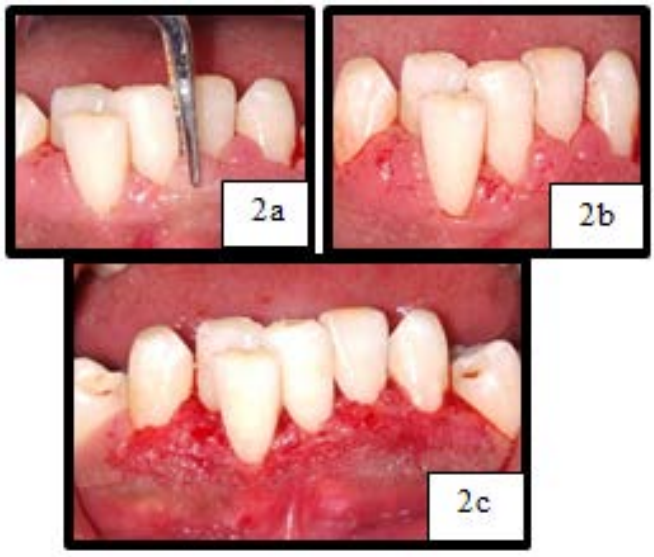


Figure1: Pre-surgical view

C. Treatment procedure: Oral hygiene measures were given to the patients and after patients satisfying their hygiene they were taken for the surgery (Figure1). Profound anaesthesia with 2% lignocaine HCL containing 1:80,000 adrenaline was obtained at both test and control surgical sites. The pockets were marked with a pocket marker to outline their course on each surface (Figure 2a, 2b). The gingivectomy incision was taken using scalpel having Bard Parker blade no. 11/15 at 45 degree beveled to the tooth surface, started apical to the points marking the course of the pockets and was directed coronally to a point between the base of the pocket and the crest of the bone. The excised tissue was removed by curettes, area was cleaned and closely examined for any remnants of calculus or granulation tissue to be removed (Figure 2c). Gingivoplasty was performed as indicated to create the physiologic gingival contours.



D. Preparation of PRF

After the recipient site preparation was completed, PRF preparation was done based on Dohan et al.[12] (3000 rpm for 10 minutes) protocol. 10 ml of venous blood was drawn in a 5 ml of two sterile tube without an anticoagulant (Figure 3a). Both the tubes was placed opposite to each other for balancing in the centrifugal machine and centrifuged immediately (Figure 3b). The resultant product consisted of three layers. a) Platelet poor plasma supernatant b) Platelet rich fibrin in the middle layer c) Red blood cells at the bottom (Figure 3c). After centrifugation, the PRF clot was obtained, separated from the RBC base using scissors, and placed in PRF box (Figure 3d, 4a). The PRF membrane was prepared by placing the PRF clot on the grill in the PRF box and covered with the compressor cover for 1 minutes to squeeze out the fluid (Figure 4b). Then the membrane was taken up from the PRF box using tweezer and placed on the exposed gingival wound taking care that it should be limited to the test site (Figure 4c). Following this non-

eugenol periodontal dressing (Coe-Pak) was applied over both control and test sites (Figure 4d). Post-operative instructions were given and patients were asked to avoid brushing at the surgical sites for at least 7 days. Antibiotic (amoxicillin with clavulanic acid 625 mg, tid) for five days, analgesic (diclofenac sodium and paracetamol tablets, SOS and 0.2% chlorhexidine gluconate mouthwash twice daily for 14 days were prescribed.

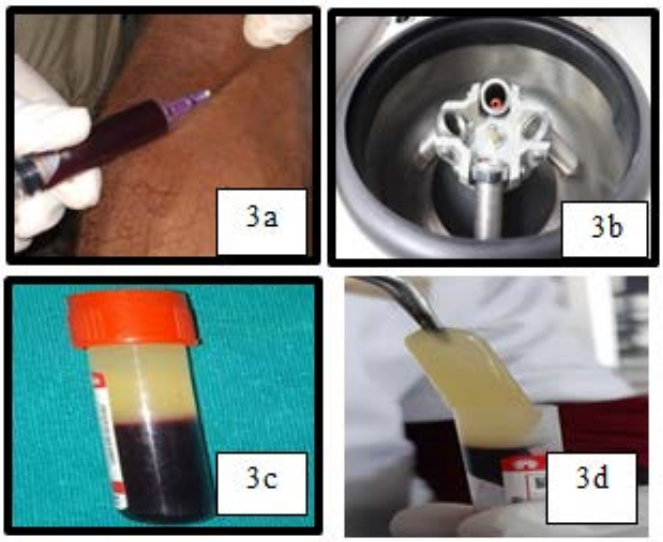


Figure 3a-d : PRF preparation

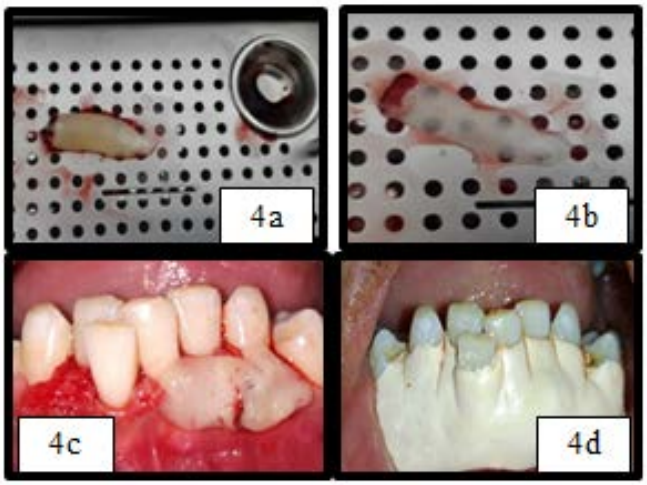


Figure 4a- d : PRF membrane preparation and its application

Post-surgical measurements: Pain response (visual analogue scale) on 1st, 3rd, 5th, 7thpost-operative days were recorded by the patients on the supplied form. Healing response (Landry and turnbull, 1988)[13] on 5th, 7th, 15th, 30th post-operative days was assessed by another operator who was unaware of control or test sites (Figure 5a, 5b).

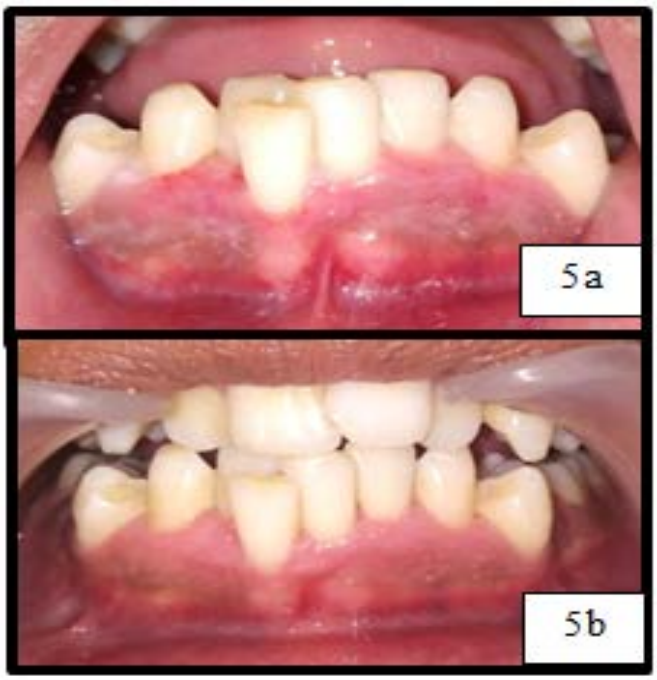


Figure 5: Post-operative view. 5a) at 5th day, 5b) at 30th day

Statistical analysis- The statistical software SPSS version 24.0 was used in the analysis. P-value less than 0.05 was considered as significant at 95% confidence level. Statistical tests applied were as follows- 1. Quantitative variables are compared using Independent t-test/Mann-Whitney test to compare mean values between the two groups. 2. Paired t-test was applied to see the relative change with respect to time. Pain index (VAS): On intragroup comparison between 3rd and 5th post-operative days, test group additionally showed statistically significant pain reduction (p=0.015) compared to control group (p=0.081) between the same time period (Table 1). However, intergroup comparison for VAS showed statistically insignificant difference at all time intervals (Table 2). Healing index: The mean scores of healing index were better in control group than test group on 5th

post-operative day but they were better in test group than control group on 7th and also on 15th post-operative day (Table 3). On 30th post-operative day, mean value scores were same in both test and control group, reflecting complete healing. However, intergroup comparison showed statistically insignificant difference at all time intervals for healing indices (Table 4). IV. DISCUSSION According to the present study, in both test and control groups, the post-operative pain got reduced progressively and till 5th post-operative day pain was mild. However, pain almost completely resolved on 7th post-operative day in both groups. Out of 10 subjects , 9 had no pain at all on 7thday, only 1 subject scored 1 on VAS on 7th day for both the groups. As, after surgical gingivectomy, the gingival wound is large, having exposed raw connective tissue surface with exposed nerve endings that induces post-operative pain.[14]

Table 1: Intragroup comparison of mean and standard deviation (SD) of VAS in control group and test group between different time intervals

Pair	Days	N	VAS Mean ± Std. Deviation (Control)	P-value (Control)	VAS Mean ± Std. Deviation (Test)	P-value (Test)
Pair 1	Day 1	10	2.90±2.55	0.091	2.70±2.35	0.266
	Day 3	10	1.40±0.51		1.70±0.94	
Pair 2	Day 3	10	1.40±0.51	0.081	1.70±0.94	0.015
	Day 5	10	1.10±0.31		1.20±0.63	
Pair 3	Day 5	10	1.10±0.31	0.000	1.20±0.63	0.001
	Day 7	10	0.10±0.31		0.10±0.31	
Pair 4	Day 1	10	2.90±2.55	0.058	2.70±2.35	0.091
	Day 5	10	1.10±0.31		1.20±0.63	
Pair 5	Day 3	10	1.40±0.51	0.000	1.70±0.94	0.001
	Day 7	10	0.10±0.31		0.10±0.31	
Pair 6	Day 1	10	2.90±2.55	0.004	2.70±2.35	0.004
	Day 7	10	0.10±0.31		0.10±0.31	

Table 2: Intergroup comparison of mean and standard deviation (SD) of VAS score at different time intervals

DAYS	Group	N	Mean ± Std. Deviation	p-value
Day 1	Test	10	2.70 ± 2.35	0.858
	Control	10	2.90 ± 2.55	
Day 3	Test	10	1.70 ± 0.94	0.391
	Control	10	1.40 ± 0.51	
Day 5	Test	10	1.20 ± 0.63	0.660
	Control	10	1.10 ± 0.31	
Day 7	Test	10	0.10 ± 0.31	1.000
	Control	10	0.10 ± 0.31	

Table 3: Intragroup comparison of mean and standard deviation (SD) of healing scores in control group and test group between different time intervals

Paired sample statistics

Pair	Days	N	Mean ± Std. Deviation (Control)	P-value (Control)	Mean ± Std. Deviation (Test)	P-value (Test)
Pair 7	Day 5	10	2.80±0.78	0.011	2.70±1.05	0.004
	Day 7	10	3.60±0.96		4.10±0.87	
Pair 8	Day 7	10	3.60±0.96	0.001	4.10±0.87	0.003
	Day 15	10	4.80±0.42		4.90±0.31	
Pair 9	Day 15	10	4.80±0.42	0.168	4.90±0.31	0.343
	Day 30	10	5.00±0.00		5.00±0.00	
Pair 10	Day 5	10	2.80±0.78	0.000	2.70±1.05	0.000
	Day 15	10	4.80±0.42		4.90±0.31	
Pair 11	Day 7	10	3.60±0.96	0.001	4.10±0.87	0.010
	Day 30	10	5.00±0.00		5.00±0.00	
Pair 12	Day 5	10	2.80±0.78	0.000	2.70±1.05	0.000
	Day 30	10	5.00±0.00		5.00±0.00	

Table 4: Intergroup comparison of mean and standard deviation (SD) of healing scores at different time intervals

T- Test

DAYS	Group	N	Mean± Std. Deviation	P-value
Day 5	Test	10	2.70 ± 1.05	0.813
	Control	10	2.80 ± 0.78	
Day 7	Test	10	4.10 ± 0.87	0.241
	Control	10	3.60 ± 0.96	
Day 15	Test	10	4.90 ± 0.31	0.556
	Control	10	4.80 ± 0.42	
Day 30	Test	10	5.00 ± .00[a]	NA
	Control	10	5.00 ± .00[a]	

a. t cannot be computed because the standard deviations of both groups are 0.

Bradykinin, a major plasma protease present during inflammation, increases vessel permeability and stimulates nerve endings to cause pain.[15] However, post-operative pain gradually decreases as the epithelization process begins over the connective tissue bed, which usually takes 5-14 days for complete epithelization.[16] On intragroup comparison between 3rd and 5th post-operative days, test group showed statistically significant pain reduction

(p=0.015) compared to control group (p=0.081) between the same time period. This could be possibly explained by the effect of PRF as it forms a dense fibrin network with leukocytes, cytokines, structural glycoproteins and also growth factors that are released from 1st day which favor matrix remodeling and early epithelization during wound healing.[17] The release of vascular endothelial growth factor (VEGF), IL-4, an anti-inflammatory cytokine found in PRF, modulates inflammation and pain by inhibiting matrix metalloproteinases (MMPs) and neutralizing transduction pathways from IL-1β, TNF-α and prostaglandins.[18-20] However, on comparing post-operative mean values of VAS score between test group and control group the results were found to be statistically insignificant at all-time intervals (p>0.05). For the healing index, on intergroup comparison between test group and control group the results were statistically insignificant (p>0.05) at all-time intervals. However, numerically mean scores of healing were better in control group than test group on 5th post-operative day (2.80±0.789, 2.70±1.059 respectively), but they were better in test group than control group on 7th (4.10±0.876, 3.60±0.966, respectively) and also on 15th post-operative day (4.90±0.316, 4.80±0.422, respectively). On 30th post-operative day, mean value scores were same in both test and control group i.e. 5.00±0.000, reflecting complete healing. Guler et al. [21] in his study performed scalpel gingivectomy and reported better epithelization at initial 7th post-operative day and complete epithelization at 14th day post-operatively, which was similar to the present study for control group. For the test group, the results were in consistent with the case reported by Priyadarshini et al.[22] where PRF application was done after gingivectomy and uneventful healing was found. One of the studies showed constant and steady release of six growth factors could be appreciated from PRF group upto 23rd day.[23] Platelet derived growth factor (PDGF) showed gradual increase in concentration from the 1st day (164.3 pg) to 183.1 pg at 23rd day. Vascular endothelial growth factor showed its peak concentration at 17th day i.e. 233.9 pg. Similarly other growth factors such as FGF, IGF, EGF, TGF also showed slow and steady release from PRF.[23] The slower release of growth factors over time is due to the ability of the fibrin matrix to store the proteins within its fibrin mesh as well as the cells capability to further release the growth factors into their surrounding microenvironment. The dynamic VEGF is critical for neo-angiogenesis during the wound healing and also facilitate in maintaining the integrity of endothelial cell lining of the blood vessel.[24] The FGF and EGF are known to play an important role in the regulation of ectodermal and mesenchymal derived cell along being a potent chemotactic and mitogenic actions.[25] Thus, the diverse action of the growth factors forms a key player in wound healing and regeneration.[26] After extensive research not many published cases were found that reported application of PRF after gingivectomy. However the following study reported the use of PRF on exposed connective tissue after gingival depigmentation procedure. Bansal et al.[27] and Dahiya et al.[28] performed gingival depigmentation procedure and reported that better healing was observed at 3rd day and 5th day post-operative in PRF sites as compared to Coe-Pak alone sites. The limitations of the present might be no histological analysis was done, which would have helped in more confirmatory results, a larger sample size should have been taken, visual analogue scale was taken for pain scores that is subjective which may vary or may not be accurately assessed by the patients, instead of sterile plastic tubes, titanium tubes should have been used for PRF preparation as these tubes have shown organized and thicker fibrin network that may promote better healing, retention of the PRF membrane should

have been carried out to prevent dislodgement from the site. For the future directions, larger sample size and use of more advanced form of platelet concentrate could enhance the soft tissue healing and less patient discomfort, for more accurate results, histological analysis should be done, newer retentive device could be used in future for better retention and long term release of growth factors from PRF.

Conclusion

The results from the present study signifies that the growth factors released from PRF at the sites might have some additional role and it can be safely concluded that PRF may be used in soft tissue wound dressing where exposed connective tissue is present to achieve uneventful healing. Further studies with more sample size should be carried out to evaluate the clinical effectiveness of PRF on healing after gingivectomy.

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