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# Recession coverage using modified coronally advanced flap with and without leukocyte & platelet-rich fibrin: A Histologic & Histomorphometric Study

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## Abstract

**Background:** Platelet rich fibrin (PRF) has been used successfully for management of gingival recession. However, there is no literature assessing the histological evaluation of PRF when used for recession coverage. Hence, the aim of this study was compare the clinical and histological efficacy of coronally advanced flap with or without PRF for management of Miller's Class I or II gingival recession.

**Materials & Methods:** A total of 18 sites with at Millers class I/II gingival recession were divided randomly into control site and test site and managed with the modified coronally advanced flap technique alone (control) or with PRF (test) in a split mouth design. Clinical variables including width of keratinized gingiva (WKG), probing depth (PD), vertical recession depth (VRD) and clinical attachment level (CAL) were recorded before surgery and at 3rd and 6th month postoperative. At the 3rd month a tissue biopsy was harvested and subjected to descriptive histological analysis.

**Results:** The pre & post-operative clinical data obtained after intergroup comparison were found to be statistically not significant. Upon histological examination, the mean thickness of the epithelium obtained in the test group was

significantly higher than that of the control group (p=0.003). Also, the test group had significantly higher amount of mature collagen (p<0.05) and higher number of cases with very dense type collagen as compared to that of the control group.

**Conclusion:** The use of PRF for Miller's class I/II recession resulted in significant increase in thickness of the epithelium and increased maturation and density of the collagen fibers.

**Keywords:** Coronally advanced flap, Gingival recession, Histological analysis, Platelet rich fibrin

**Introduction**

Gingival recession is the root exposure caused due to the apical migration of gingival margin. This may lead to compromised aesthetics & plaque control, dentin hypersensitivity, and increased risk for root caries.[1] Since past few decades, several techniques have been employed to cover the denuded root surfaces. Connective tissue graft with coronally advanced flap has been considered as the gold standard for root coverage.[2] However, this technique has certain drawbacks such as increased morbidity due to second surgical site and limited amount of the graft that can be harvested.[3] Hence, the search for superior alternatives continues till date.

Platelet rich fibrin is a completely autologous platelet concentrate which is increasingly finding application in dentistry. It contains various growth factors such as platelet derived growth factor, vascular endothelial growth factor, transforming growth factor which provides enhanced healing at the site. It acts as an immune modulating node and plays a beneficial role in mitogenesis and wound recolonization. It has an easy single step protocol, is cost effective and provides a steady release of growth factors as compared to PRP.[4] Several studies have demonstrated the application of PRF for recession coverage.[5-7] A recent randomized controlled trial demonstrated that the efficacy of PRF with coronally advanced flap is same as that of connective tissue graft as measured in terms of percentage root coverage and concluded by stating that PRF can be used as an alternative to connective tissue graft for recession coverage.[6]

However, in our comprehensive literature search, we found no studies which have assessed histologic evaluation of platelet rich fibrin when used for recession coverage. Hence, This study was done with the objective to assess and compare the clinical and histological efficacy of coronally advanced flap alone as compared to coronally advanced flap with PRF for management of Miller's Class I or II gingival recession.

**Materials and methods**

Thirteen systemically healthy patients age range of 20-45 years with Miller's Class I or II gingival recession on the facial aspect of the anterior teeth were selected for the study from the out-patient department of department of Periodontology. Subjects with poor oral hygiene, pregnant females and smokers were excluded from the study. The study was performed according to the guidelines of the Declaration of Helsinki and was approved by the Institutional Ethical Committee. All the patients were explained about the surgical procedure and the written consent was taken prior to the study.

**Clinical parameters**

A total of 26 sites were randomly divided into the control site (CAF only) and the test site (CAF with PRF) using a coin toss method (n=13 each) in a split–mouth design technique. Clinically, width of keratinized gingiva (WKG), Probing depth (PD), clinical attachment level (CAL), and vertical recession depth (VRD) were recorded for all the selected sites. All the measurements were recorded by a single examiner with the help of customized acrylic stents and UNC-15 periodontal probe

at the baseline, 3 month and 6 months postoperatively for both the groups. Phase I therapy was performed for all the selected subjects prior the surgical procedure.

**Surgical technique**

All the surgeries were performed by a single examiner. After obtaining adequate anaesthesia using 2% lignocaine hydrochloride containing 1:100,000 epinephrine, two oblique incisions (extending 3mm each in the mesio–distal direction) at a distance from the vertex of the anatomic papilla equal to the depth of the recession; and two bevelled vertical-releasing incisions extending up to the alveolar mucosa were made. A split-full-split thickness flap was elevated and the root surface was debrided mechanically with the help of curettes.[8] The remaining buccal soft tissue of the anatomic interdental papillae was de-epithelized. Following this, in the test group, 10 ml blood was collected from the ante-cubital vein without anticoagulant and immediately centrifuged at 2700 revolutions per minute for 12 minutes (L-PRF).[4] The fibrin clot thus obtained was compressed to form a membrane which was then positioned over the recession defect, just apical to the cemento-enamel junction (CEJ) and the flap was coronally displaced 2 mm coronal to cementoenamel junction and sutured (Figure 1a-d). In the control group, only coronal repositioning of the flap was performed and then suturing was done. Postoperative analgesics were prescribed as required. Patients were instructed not to brush their teeth in the treated area, but to rinse their mouth with chlorhexidine solution (0.12%) twice daily for 1 min. Patients were recalled after 2 weeks for suture removal. After this, they were instructed to use a soft toothbrush with roll technique and were placed on maintenance therapy.

**Tissue collection and histologic analysis**

At the end of 3 months, all clinical parameters were recorded following which a tissue sample was collected from both the test and the control site of each patient under local anaesthesia. A specimen containing both epithelium connective tissue was harvested at-least 2mm away from the gingival margin in the attached gingiva from the mid portion of the healed site measuring around 2mm x 2mm (Figure 2).[9-11] The biopsy wound was covered with collagen sponge to facilitate healing (Collatape, Zimmer dental, USA). All the specimens obtained were immersed in 10% neutral buffered formalin solution for fixation at room temperature for one day. Following this, the specimen was dehydrated in serially graded alcohol. Then paraffin embedding was done and the tissue was then sectioned slices of thickness of 4μm each(Leica RM 2155). The specimens were stained by means of standard haematoxylin and eosin stain for epithelial surface characteristics and with picrosirius red stain for connective tissue analysis (collagen fibres).[10,11] The specimens were examined at 40x magnification using research microscope and image analysis software. The parameters examined in the epithelium were type of keratinization (orthokeratinization or parakeratinization) and the thickness of epithelium (in μm) whereas in the connective tissue, maturation of collagen fibres and density of collagen fibres were assessed.[11]

All the clinical and histological parameters obtained were drawn in a standard proforma and subjected to statistical analysis. The statistical analysis for inter-group comparison of the clinical parameters was obtained by using Mann Whitney U test. A statistical difference of $p<0.05$ was considered to be statistically significant. Statistical analysis was done using SPSS software version 17.0.

**Results**

All the patients completed the stipulated follow and there were no drop outs from the study. The pre & post-operative clinical data obtained after intergroup

comparison was found to be statistically not significant (Table-1). The vertical recession depth in the control and test group were 3.00 ± 0.87 & 3.78 ± 1.07 at baseline and reduced to 0.33 ± 0.50 and 0.49 ± 0.60 respectively at the end of 6 months ($p>0.05$).

Upon histological examination, the mean thickness of the epithelium obtained in the control and the test group were 359.1± 88.2 and 447.2± 84.6 respectively (figure 3a,3b). The results obtained were statistically highly significant ($p=0.003$). The results gained after connective tissue analysis is enumerated in table 2. When the amount of mature collagen was compared between the control and test group, the test group had significantly higher amount of mature collagen ($p<0.05$). Also, most of the mature fibres in test group were of very dense type (61.1%) as compared to that in control group where the fibers were of dense, moderate or loose type (figure 4a,4bTable-2).

Discussion

Coronally advanced flap in conjuction with PRF for management of gingival recession has demonstrated good results in several recent studies. Its advantages include predictable root coverage, good colour blend with adjacent soft tissues and no donor site morbidity.[5-7] Even though several studies have demonstrated equal or superior results with PRF, no studies have assessed the histologic changes with the use of PRF. Hence, the present study compared the clinical and histological efficacy of coronally advanced flap alone as compared to coronally advanced flap with PRF for management of Miller's Class I or II gingival recession. A split mouth design was selected because it excludes the influence of patient specific characteristics and facilitates the interpretation of trials by minimizing the effects of inter-patient variability. The selected sites were randomly divided into control site and test site to avoid any site specific bias.

The clinical data including probing depth, width of keratinized gingiva, vertical probing depth and clinical attachment level measured at the baseline, one month and three months post-operatively. Width of keratinized gingiva, vertical probing depth and clinical attachment level all demonstrated a significant improvement in both the groups when compared from baseline to three months. This indicates the success of both the treatment groups in management of the recession. However, the intergroup differences were not statistically significant. These findings are in agreement of those of Kuka & co-workers who found no additional clinical benefits of using PRF membranes except increase in tissue thickness.[7] This indicates that clinically, there is no difference in the results obtained for management of Miller's Class I/II gingival recession using CAF with or without addition of PRF.

In histological analysis, the intergroup comparison of mean thickness of the epithelium revealed significantly superior results in the test group. This can be attributed to the superior fibrin matrix provided by PRF which guides the cells into the wound.[12-15] The tissue sections observed using Picrous serous red stain revealed more maturation and density of the collagen fibres in the test (PRF) group as compared to the control group. The maturation of collagen fibres in the test group was 100 % while that in the control group was 72%. Also the mature collagen fibres in the test group were of the category very dense and dense while that of the control group were of the category loose and moderate. In a recent study, it was found that PRF significantly increased the release of Collagen-related proteins HSP47 and LOX compared with untreated controls in human osteoblasts($p<0.05$).[16] Also, PRF was seen to affect the proliferation, migration and collagen expression of collagen-1 in culture as compared to control. These studies can justify our

findings of denser and more mature collagen fibres in PRF group. PRF enhances soft tissue healing, promotes initial stabilization, and revascularization in various soft and hard tissue procedures.[17]

The key reason for the superior result was the healing potential of PRF. Our findings can be attributed to the presence of growth factors such as platelet derived growth factor, vascular endothelial growth factor, transforming growth factor present in PRF membrane. PDGF and VEGF, the main autologous growth factors of PRF, are associated with pericytes and capillary formation, respectively, and serve as potent angiogenic factors.[4,15-17] These growth factors might enhance soft tissue healing by increasing the angiogenesis and matrix biosynthesis during early wound healing. The increased thickness of epithelium and collagen fibre density may translate clinically into increased tissue thickness as observed by Kuka & co-workers.[7] It has been hypothesized that an increase in gingival thickness, as obtained with tissue grafting, will help prevent future recession in patients with a thin periodontal phenotype and provide a more stable result clinically.[18-20] Also, PRF application may prove beneficial for patients with thin biotype as demonstrated recently.[21]

However, further studies with larger sample size and long term evaluation are necessary to confirm the constancy of the results and the histological effectiveness of autologous PRF membrane when used for recession coverage.

**Conclusion**

On the basis of histological results of the study, it can be concluded that the combined approach of using CAF and PRF for treatment of Miller's class I/II gingival recession yields an increased mean thickness of the epithelium, significantly higher amount of mature collagen and very dense type collagen.

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Table 1

| | | Control group | Test group | P value* |
|---|---|---|---|---|
| Width of keratinized gingiva | Baseline | 2.89 ± 0.78 | 2.67 ± 0.71 | 0.57, NS |
| | 1 month | 3.44 ± 1.01 | 3.56 ± 0.73 | 0.70, NS |
| | 3 month | 3.56 ± 1.01 | 4.00 ± 1.00 | 0.39, NS |
| Probing depth | Baseline | 1.44 ± 0.53 | 1.56 ± 0.53 | 0.68, NS |
| | 1 month | 0.83 ± 0.25 | 0.78 ± 0.26 | 0.67, NS |
| | 3 month | 0.72 ± 0.26 | 0.78 ± 0.26 | 0.68, NS |
| Clinical attachment level | Baseline | 4.44 ± 0.88 | 5.22 ± 0.97 | 0.11, NS |
| | 1 month | 1.17 ± 0.66 | 1.67 ± 0.79 | 0.23, NS |
| | 3 month | 1.11 ± 0.65 | 1.56 ± 0.81 | 0.25, NS |
| Vertical recession depth | Baseline | 3.00 ± 0.87 | 3.78 ± 1.07 | 0.22, NS |
| | 1 month | 0.33 ± 0.50 | 0.89 ± 0.60 | 0.65, NS |
| | 3 month | 0.44 ± 0.53 | 0.78 ± 0.67 | 0.52, NS |

NS: non-significant

Figure legends

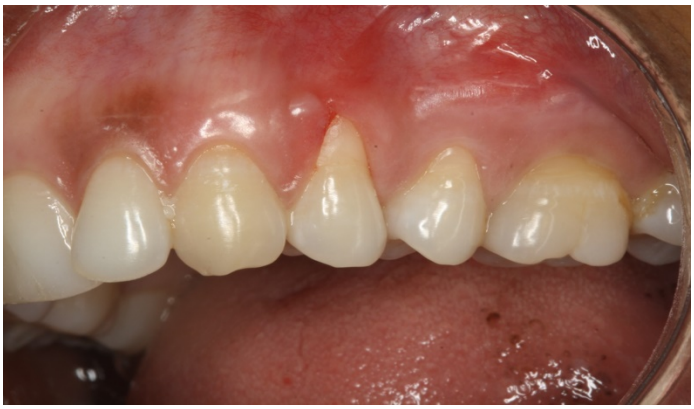

Figure 1: a) Pre-operative picture depicting recession.

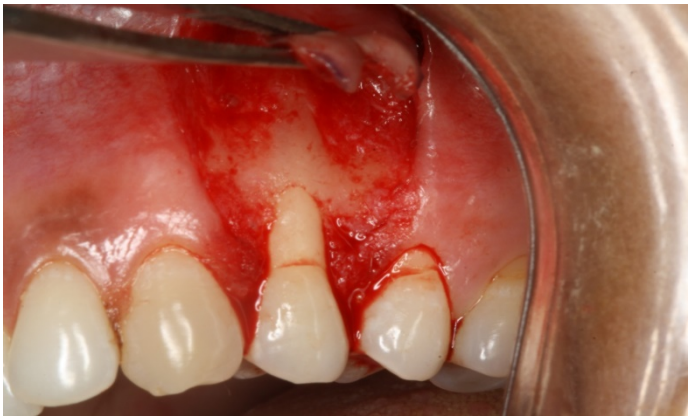

Figure 1: b) Preparation of recipient site

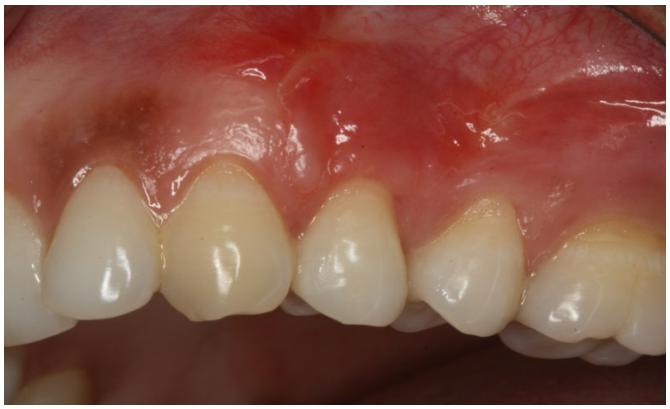

Figure 1: c) Tissue biopsy taken for histologic analysis

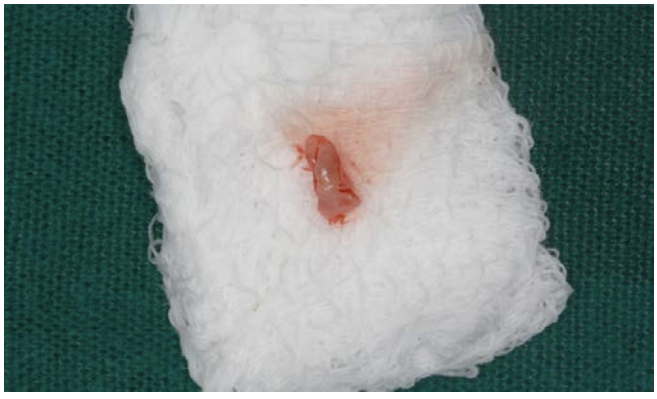

Figure 1: d) 6 month post-operative view.

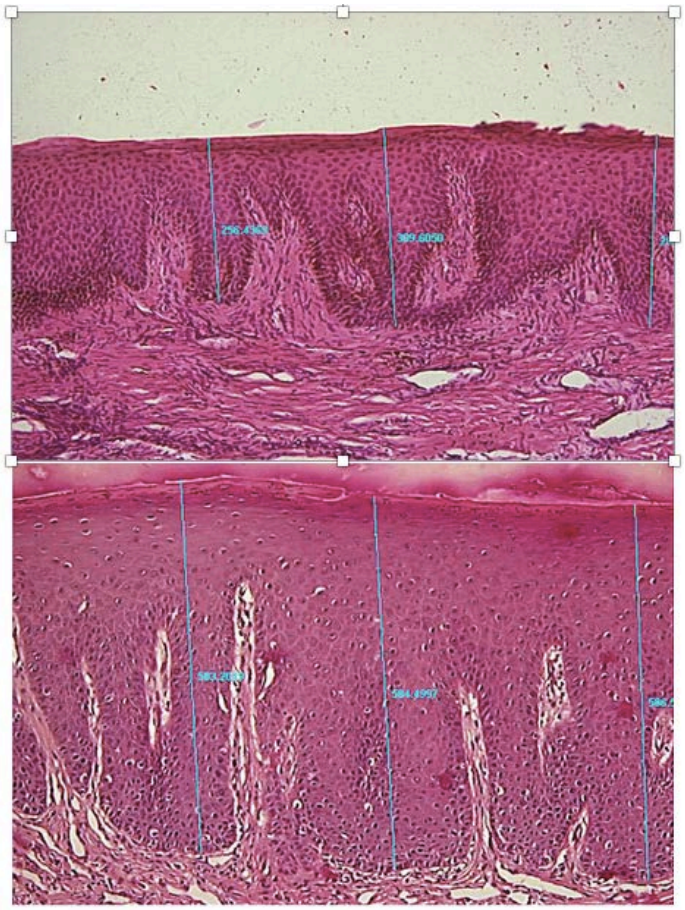

Figure 2: a) H & E stained section in control group b) H & E stained section in test group

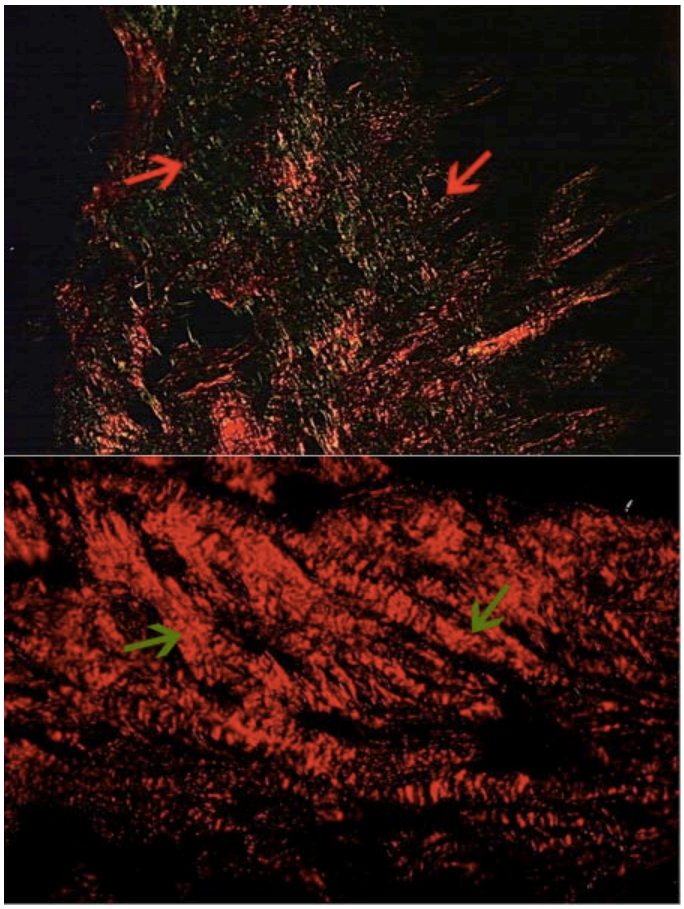

Figure 3: a) Picrosirius red stained section in control group b) Picrosirius red stained section in test group