Comparative Evaluation of Coronally Advanced Flap Procedure in Conjunction with Amniotic Membrane Versus Coronally Advanced Flap with Platelet-Rich Fibrin Membrane in Patients with Miller’s Class I and II Gingival Recession Defects

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Abstract

Introduction Amnion membrane (AM) and platelet-rich fibrin (PRF) have been used as Guided Tissue Regeneration (GTR)-based root coverage procedures in treatment of gingival recession. The objective of the present study was to comparatively evaluate the coronally advanced flap (CAF) procedure in conjunction with AM versus CAF with PRF in patients with Miller’s class I and II gingival recession defects (GRD).

Methods The sample size (Miller’s Class I and Class II GRD) consisted of 24 patients who were stratified into two groups randomly (12 for each group). Group A patients were treated by CAF with PRF and Group B patients were treated by CAF with AM. Clinical parameters comprising plaque index (PI), gingival index (GI), pocket probing depth (PPD), clinical attachment level (CAL), and GRD were assessed at baseline, 3 months, and 6 months postoperatively.

Results The percentage of root coverage obtained in the study groups was 62% and 77%, respectively, for Groups A and B. Statistically significant difference was obtained in the clinical parameters (PPD, CAL, and GRD) of Group B, which was treated by CAF with AM.

Conclusions Significant difference was found between Group A (CAF+ PRF) and Group B (CAF+ AM) membrane treated sites in reduction of PPD, gain in clinical attachment level, reduction of GRD from baseline to 3 months and baseline to 6 months, with more significant results seen with Group B.

Keywords
- amniotic membrane
- coronally advanced flap
- platelet rich fibrin membrane

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Introduction

Gingival recession as defined by the term Glossary of Periodontal Terms (GPT) is a gingival margin apical to Cemento-enamel junction (CEJ) that causes aesthetic and functional problems such as hypersensitivity in root, cavity formation in root and cervical abrasions in patients.1

Various techniques such as the laterally positioned flap strategy, free gingival graft method, connective tissue method, free gingival graft/coronally positioned flap method, and a cellular dermal matrix method have been used over the years to treat gingival recession. With coronally advanced flap (CAF) technique, we achieved optimum root coverage with good color mixing and complete recovery marginal morphology of soft tissue as it was originally.

Root implantation using GTR results are similar as traditional root coverage procedures, but also reflects the formation of new histological attachment formation.2 Platelet-rich fibrin (PRF) is a second generation of autologous platelet concentrate comprising platelets, cytokines, leukocytes, various growth factors, and circulating stem cells. As a supplement to CAF, the beneficial effects of PRF on gingival recession defect (GRD) have been clarified with promising results.3 However, certain limitations such as the requirement for abstinence from blood and time-consuming procedures have attracted medical attention for allografts such as amniotic membrane (AM).

AM is a tissue derived from the placenta that combines the pluripotent cellular feature with many growth factors and proteins that aid in the rapid healing of gingival wound and regeneration of periodontal tissues.

Materials and Methods

A total of 24 sites with Miller’s Class I and II gingival recession were selected from healthy patients without any systemic illnesses between the ages of 20 and 60 years, regardless of economic, religious, and sexual orientation and who reported in the Department of Periodontology, Faculty of Dental Sciences, PDM University, Bahadurgarh, Haryana.

Selection Criteria

Patients with systemic health with Miller’s Class I and II gingival recession greater than or equal to 2 mm depth and width, sufficient vestibular depth, sufficient keratinized gingival width, no history of allergies, willing to follow recommended plaque control and follow-up regimen, patients those who had not taken antibiotics in the last 6 months were included in the study.

The exclusion criteria included patients who had a history of any surgical procedure in a designated area 6 months ago, smokers and tobacco chewers, systemic diseases or at-risk, on antibiotics 6 months prior to treatment, pregnant and lactating women, undergoing orthodontic therapy.

Selected Sites were Randomly Assigned to the Following Research Groups

Study Groups
GROUP A: CAF + PRF
GROUP B: CAF + AM

Preoperative Phase

Surgical procedures included phase I treatment that included scaling and root planing. Oral hygiene instructions were given and patients were called after 2 weeks for further examination. Patients who showed satisfactory response were considered in the study. In all selected patients, radiographic and blood tests were performed. Selected patients had little or no loss of radiographic bone in the area between the teeth.

Group A (CAF + PRF Group)

PRF Membrane Preparation (Fig. 1)

PRF is an autologous compound of platelets in the plasma. It is a second-generation platelet concentrate widely used to accelerate the healing of soft and hard tissues. Choukroun et al4 in 2001 developed PRF in France. The PRF in our study was formed in accordance with a protocol developed by Dohan et al.5 Intravenous blood was collected in a sterile 10 mL tube without anticoagulant and immediately incorporated into a centrifugation machine at a rate of 3,000 revolutions (approximately: 400 g/min for 10 minutes. After centrifugation, the product was made into three layers. The uppermost layer consisted of acellular PPP (platelet-poor plasma), the PRF clot was present in the middle and RBCs were observed to be housed at bottom of a test tube. PRF clot was collected using a sterile tweezer and scissor after PPP removal. The clot was then transferred to a sterile gauge piece that was pressed between two slabs of sterile glass, allowing it to turn into a shape of membrane.

Group-B (CAF + AM Group)

Amniotic Membrane (Fig. 2)

This allograft of soft tissue contains many layers of dehydrated amnion tissue processed by Purion, which is a technology for processing tissue that protects the natural structure of the amnion while purifying and binding its layers together. As per the manufacturer’s intervention, any sides of available bioreosorbable AM components may contact the tooth surface and the other can be in contact with elevated flap. The same surgical procedure as described below was followed. The commercial AM (1 × 1 mm) was cut into the required shape and length with scissors and placed in place of the recession.

Surgical Phase

The patient was asked to rinse before surgery with 10 mL of 0.2 chlorhexidine diluted solution. The selected site was locally administered using 2% xylocaine HCl adrenaline (1:
80,000) before starting the procedure. All selected cases underwent root coverage procedure by CAF. Prior to giving incisions, in Group I (CAF and PRF), patient blood samples were taken by the assistant to prepare the PRF. Two basic horizontal incisions were made in mesial and distal directions from CEJ up to 1 mm beyond the proximal line angle of adjacent teeth leaving the interdental papilla intact (Fig. 1B, 2B). Two vertical releasing incisions were given on the involved tooth interdentally on labial aspect that extended beyond the mucogingival junction and entered the mucobuccal fold until the point where the flap could be placed over the actual element without tension. (Fig. 1C, 2C). Sulcular incisions using BP blade no. 12/15 were made and a trapezoidal mucoperiosteal flap was raised with a blunt
dissection. In the apical area to the recession defect, partial thickness dissection was performed (► Figs. 1D, 2D). The buccal portion of the intact papilla was de-epithelialized to serve as a recipient of connective tissue for a coronally advanced repositioned flap. Care was taken to preserve interproximal tissue as much possible. The root planing of exposed roots was done using universal curette 2R/2L and or 4R/4L (Hu-Friedy). The autologous PRF membrane for Group A patients (► Fig. 1E) or Freeze Dry, Irradiated AM for Group B patients (► Fig. 2E) was cut and placed to cover the bony recession defect extending from CEJ to cover the adjacent bone, mesially, distally, and apically by 2 to 3 mm. The buccal flap was repositioned to cover the membrane and the CAF was kept in place with 4–0 unbleached silk (► Fig. 1F). The gingival flap margin was repositioned on the enamel of the tooth at both test and control sites and was held in place by a horizontal sling suture. Interrupted sutures were placed to close the vertical releasing incisions with the same suture material. Non-eugenol (Coe-Pack) periodontal dressing was placed (► Fig. 1G, 2F). Patients were given systemic antibiotic Cap. amoxycillin 500 mg (tds) for 5 days and Tab. ibuprofen (bd) for 3 days and then SOS. Patients were instructed to rinse with chlorhexidine digluconate (0.2%) twice a day orally every 2 weeks and they were asked to avoid brushing or flossing, the practice of cleaning for 1 week. Patients were asked to report 10 days after surgery to remove the pack and earlier in case of a serious incident.

Results
Collected data were statistically analyzed. Based on data distribution, unpaired t-tests, analysis of variance (ANOVA) tests for repeated steps, post-hoc Bonferroni tests were used to obtain results.
Periodontal clinical parameters such as plaque index (PI), gingival index (GI), PPD, relative clinical attachment level (RCAL), GRD, root coverage (RC), were recorded 3 months and 6 months after Phase 1 treatment. The percentage of RC obtained from the study groups was 62% and 77%, respectively, in Group A and B. Significant statistical differences were found in the clinical parameters (PPD, RCAL and GRD) of Group B treated by CAF with AM (►Table 1, 2 and ►Table 3).

### Table 1  Periodontal probing depth

<table>
<thead>
<tr>
<th></th>
<th>Group A (PRF)</th>
<th>Group B (Amnion membrane)</th>
<th>Mean difference</th>
<th>t-test Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Baseline</td>
<td>1.6667</td>
<td>.49237</td>
<td>2.0000</td>
<td>.42640</td>
<td>−0.333</td>
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<tr>
<td></td>
<td>−1.773</td>
<td>0.09</td>
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<td></td>
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<tr>
<td>3 months</td>
<td>1.3333</td>
<td>.49237</td>
<td>1.0000</td>
<td>.00000</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td>2.345</td>
<td>0.039*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.3333</td>
<td>.49237</td>
<td>1.0000</td>
<td>.00000</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td>2.345</td>
<td>0.039*</td>
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</table>

Unpaired t-test, *Significant difference.

Intergroup comparison of mean PPD at baseline, 3 months, and 6 months between PRF group and amnion membrane group was done using the unpaired t-test. At baseline, no statistically significant difference (p > 0.05) could be detected among PRF group and amnion membrane group regarding mean PPD. While at 3 months as well as 6 months, the mean PPD among PRF group was found to be significantly (p < 0.05) higher than that among the amnion membrane group.

### Table 2  Clinical attachment loss

<table>
<thead>
<tr>
<th></th>
<th>Group A (PRF)</th>
<th>Group B (Amnion membrane)</th>
<th>Mean difference</th>
<th>t-test Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Baseline</td>
<td>5.5833</td>
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<td>5.7500</td>
<td>0.86603</td>
<td>−0.1667</td>
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<tr>
<td></td>
<td>−0.462</td>
<td>0.649</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3 months</td>
<td>3.2500</td>
<td>0.75378</td>
<td>2.5000</td>
<td>0.52223</td>
<td>0.75000</td>
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<tr>
<td></td>
<td>2.833</td>
<td>0.01*</td>
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<tr>
<td>6 months</td>
<td>2.8333</td>
<td>0.57735</td>
<td>2.0000</td>
<td>0.60302</td>
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<td></td>
<td>3.458</td>
<td>0.002*</td>
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Unpaired t-test, *Significant difference.

Intergroup comparison of mean CAL at baseline, 3 months, and 6 months between PRF group and amnion membrane group was done using the unpaired t-test. At baseline, no statistically significant difference (p > 0.05) could be detected among PRF group and amnion membrane group regarding mean CAL. While at 3 months as well as 6 months, the mean CAL among PRF group was found to be significantly (p < 0.05) higher than that among the amnion membrane group.

### Table 3  Gingival recession depth

<table>
<thead>
<tr>
<th></th>
<th>Group A (PRF)</th>
<th>Group B (Amnion membrane)</th>
<th>Mean difference</th>
<th>t-test Value</th>
<th>p-Value</th>
</tr>
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<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Baseline</td>
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<td>0.79296</td>
<td>3.7500</td>
<td>0.62158</td>
<td>0.16667</td>
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<td></td>
<td>0.573</td>
<td>0.572</td>
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</tr>
<tr>
<td>3 months</td>
<td>1.9167</td>
<td>0.66856</td>
<td>1.5000</td>
<td>0.52223</td>
<td>0.41667</td>
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<tr>
<td></td>
<td>2.101</td>
<td>0.045*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.5000</td>
<td>0.52223</td>
<td>1.0000</td>
<td>0.60302</td>
<td>0.50000</td>
</tr>
<tr>
<td></td>
<td>2.171</td>
<td>0.041*</td>
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</tbody>
</table>

Unpaired t-test.

Intergroup comparison of mean GRD at baseline, 3 months and 6 months between PRF group and Amnion membrane group was done using the unpaired t-test. At baseline, no statistically significant difference (p > 0.05) could be detected among PRF group and amnion membrane group regarding mean GRD. While at 3 months as well as 6 months, the mean GRD among PRF group was found to be significantly (p < 0.05) higher than that among the amnion membrane group.

Discussion

Gingival recession a very common issue with its effect on both the beauty and sensitivity of dentine (Hugoson et al. 2008, Röthlisberger et al. 2007). The main target of root coverage techniques is complete resolution of the recession defect, with minimal probing depth on post-treatment, as well as good chromatic integration and texture of the covering tissue with adjacent resident soft tissues.

In CAF techniques, additional tissue or materials can be inserted between the flap and the root zone, most commonly connective tissue graft (CAF + connective tissue graft), non-resorbable barriers,10 bio-resorbable barriers, enamel matrix derivatives, PRF, a cellular dermal matrix, or tissue-engineered human fibroblast-derived dermal substitute.11 Meta-analysis from a variety of systematic reviews, concluded that CAF or combined procedures had the greatest...
potential to reduce gingival recession and complete RC, thus making it a widely used method (1989).

The PI scores of the current study were consistent with Shiehet’s study using a collagen barrier to root implants showed that PI remained low and did not show significant differences during the 6-month trial period. They suggested that the collagen membrane was well-tolerated by underlying tissues and did not increase plaque accumulation or gingival inflammation. GI scores were compared between the two groups and there was no significant difference between the two groups. According to Zucchelli (2005), a decrease in GI showed that subjects maintained good oral hygiene during the study period and suggested uneventful healing as well as improved gingival health during the study. In addition, both AM and PRF membranes were well-tolerated for tissue with excellent tissue contour and color blend and did not enhance the accumulation of plaque or gingival inflammation.

The mean PPD reduction in both groups was found to be statistically significant and the results are consistent with the findings of Gupta et al. who also showed a significant reduction in PPD at 3 and 6 months after surgery in both groups. As compared with the baseline that may result from strict maintenance of the patient’s oral hygiene and subsequent reduction of gingival inflammation, it was intended to reduce GI points throughout the study period.

The gain in RCAL is significantly higher in Group B. These results are consistent with reports by Wallace et al. showing CAL gain of 2.4 mm in 4 months using the placental-derived membrane. Regarding mean reduction in GRDs, the results are consistent with a study by Rehan et al. who concluded that rate reduction in GRDs in both groups was found to be statistically significant, but AM showed a higher percentage of RC than PRF and found use bioresorbable AM to be more profitable than PRF due to the need for the preparation of an autologous PRF biomaterial.

The percentage of covered root (PRC) was found clinically significant with a greater PRC with bioresorbable AM (76.52%) than the PRF membrane (61.52%) 6 months after surgery. These results are in line with a study by Shetty et al. who proposed the use of bioresorbable AM as additive material alternate to sub-epithelial-connective tissue to reduce the need for second site for surgery and an alternate to PRF in reducing the need for preparation of the autologous biomaterial.

In the current study in Group A (CAF + PRF), the RC rate from baseline to 3 months was 51.25%, which increased to 61.52% at the end of 6 months. The PRC in our study (61.52%) is slightly smaller compared with the studies of Aleksic et al. (74.94%), Thamaraiselvan et al. (74.16), and Aroca et al. (80.71%) and Padma et al. who reported 70.73% root coverage at 3 months after surgery; however, at 6 months reported 100% root coverage. However, 100% RC was observed in the Gupta et al. study and 96% RC in the Kumar et al. study.

In the current study in Group B (CAF + AM), the mean RC from baseline to 3 months was 61.94%, which increased to 76.52% at the end of 6 months. The PRC in our study (76.52%) was less as compared with Mehta et al.’s (95%) Gurinsky’s (97%) studies; Shah et al. reported 100% RC in their study.

Therefore, within the scope of our study, we were able to note that both PRF and dehydrated AM devices were found to be effective in treating GRD of Miller’s class I and II in the CAF method. However, AM has shown better results in clinical terms and has certain advantages over PRF membranes such as better handling properties, better root adaptation, and reduced surgery time required to repair the autologous PRF membrane.

**Conclusions**

The study showed that there was a significant reduction in PPD and benefit in RCAL in both groups, with 3 and 6 months post-treatment follow-up outcomes compared with baseline data. Results were clinically significant with a larger PRC with bioresorbable AM (76.52%) than a PRF membrane (61.52%) 6 months after surgery.

**Conflict of Interest**

None declared.

**References**


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**Table 4** Percentage Root Coverage

<table>
<thead>
<tr>
<th>Root coverage (%)</th>
<th>Group A (PRF)</th>
<th>Group B (Amnion membrane)</th>
<th>Mean difference</th>
<th>t-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 3 months</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51.25 13.5424</td>
<td>61.9444 13.4809</td>
<td>10.69444</td>
<td>1.939</td>
<td>0.065</td>
</tr>
<tr>
<td>At 6 months</td>
<td>61.528 12.4002</td>
<td>76.5278 16.0563</td>
<td>15.00000</td>
<td>2.561</td>
<td>0.018</td>
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</table>

Unpaired t-test. * Significant difference.

Intergroup comparison of mean root coverage (%) at 3 months and 6 months between PRF group and amnion membrane group was done using the unpaired t-test. At 3 months, no statistically significant difference (p > 0.05) could be detected among PRF group and amnion membrane group regarding mean root coverage (%). While, at 6 months, the mean root coverage (%) among the amnion membrane group was found to be significantly (p < 0.05) higher than that among the PRF group.
CAF in Conjunction with AM Versus CAF With PRF Membrane in Patients With Miller’s Class I & II GRD

Lamba et al.

17 Wallace SC. Root coverage grafting comparing placental derived membrane to acellular dermis matrix: a case series. Dentistry 2012;2(5):1–4