Comparative Evaluation Of Coronally Advanced Flap And Platelet-Rich Fibrin Membrane With Or Without Demineralized Freeze-Dried Bone Allograft In The Management Of Isolated Gingival Recession Defects: In-Vivo Study.

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Abstract
Aim: This randomized controlled clinical trial aimed to clinically evaluate the efficacy of coronally advanced flap (CAF) and platelet-rich fibrin membrane (PRF) with or without demineralized freeze-dried bone allograft (DFDBA) in the management of isolated gingival recession defects.

Methods: A total of 20 bilateral Miller’s Class I/II recession defects were randomly treated with CAF+PRF (control group; n=10) and CAF+PRF+DFDBA (test group; n=10). Outcomes such as gingival index, plaque index, probing depth, recession depth, width of keratinized gingiva, and clinical attachment level were recorded at baseline and 6 months post-surgery. Root coverage percentage was obtained at 6 months postoperatively.

Results: Clinical parameters in both control and test groups at baseline and 6 months showed statistically significant (p<0.05) improvement in probing depth, recession depth, clinical attachment level, and increase in the width of keratinized gingiva. Clinical parameters between the CAF+PRF group and CAF+PRF+DFDBA group were compared at 6 months post-surgery and there was no statistically significant difference. (p>0.05) The percentage of root coverage at 6 months post-surgery was statistically significant (p<0.05) in both control and test groups. At 6 months post-surgery, although the root coverage was found to be better with the addition of DFDBA, there was no statistically significant difference between both groups. (p>0.05)
Conclusion: Both the control and test group sites healed uneventfully. However, the above findings are suggestive of no additional benefit of using DFDBA. The histological evaluation regarding the nature of periodontal healing at the end of 6 months would have been the most appropriate method to evaluate the intergroup comparison outcomes in this study.

Keywords: Platelet-rich fibrin, coronally advanced flap, demineralized freeze-dried bone, allograft, gingival recession.

Introduction
Gingival recession is the apical displacement of the gingival margin away from the cemento-enamel junction (CEJ) with the resultant root surface exposure.\(^1\) Its development is significantly related to mechanical factors (such as toothbrush trauma), inappropriate oral hygiene maintenance and periodontal disease along with precipitating factors like thin gingival biotype, frenum pull, buccally positioned teeth, root prominence and alveolar bone dehiscence. Untreated gingival recessions negatively influence the long-term prognosis of the involved teeth in spite of good patient motivation.\(^2\) Routinely, patients with isolated or multiple gingival recessions seek treatment when esthetics or root hypersensitivity are their concern.

Gingival recession is the most common indication for periodontal plastic surgery which includes prevention, correction, or elimination of defects that are either anatomical, developmental, or traumatic in gingiva or alveolar mucosa. Several techniques are used to attain root coverage, such as pedicle grafts, coronally advanced flap (CAF), free palatal autograft and subepithelial connective tissue graft (SCTG).\(^3\)

In terms of successful root coverage procedure, SCTG is considered to be the ‘gold standard’.\(^4\) However, it is time-consuming, there is a limited supply of donor tissue and the necessity for a donor site leads to its increased morbidity, post-operative discomfort, and post-surgical bleeding.\(^5\) Increased gingival tissue thickness has also been a predictable outcome SCTG is used in combination with the pedicle flap to cover the exposed root surfaces.\(^6\) The drawbacks inherent with autografts have instigated a search for alternative approaches.

A meta-analysis from the recent systematic review states that the percentage of root coverage with CAF procedure is 34 - 86.67%.\(^7\) Therefore, its modifications can result in improved success rate and predictability, making it the most preferred method for root coverage procedure.
variety of biologic mediators, bone substitutes and non-resorbable and bioresorbable membranes have been investigated which reported different clinical outcomes. However, none of these approaches outperformed the effectiveness of the gold standard CAF with SCTG in regards to all clinical parameters. Choukron’s platelet-rich fibrin (PRF) preparation is a second-generation platelet concentrate and an autologous leukocyte biomaterial. The natural fibrin architecture accounts for its significant cicatricial properties and provides a living-tissue scaffold that can be suggested to treat gingival recessions without the morbidity of graft harvest. Also, it has been the treatment of choice in combination with CAF, showing superior clinical outcomes.

Space maintenance between the overlying barrier and the root surface has a critical role in attaining root coverage. It permits progenitor cells’ migration over the detoxified root surface and differentiates to form cementum and periodontal ligament cells. The placement of bone graft beneath the membrane has overcome the difficulty of space maintenance as compared to membrane technique alone, thereby, allowing periodontal tissue regeneration. Currently, the widely used graft material is demineralized freeze-dried bone allograft (DFDBA). Due to its potential to create and maintain space, it may be the material of choice to be used for root coverage procedures in conjunction with non-resorbable and bioabsorbable membranes. However, there is no literature available concerning the use of DFDBA with PRF membrane to treat human gingival recession defects by using the CAF technique. Hence, the purpose of this research work was to evaluate the clinical efficacy of DFDBA (Rocky Mountain particulate allograft) in the management of isolated gingival recession defects using the CAF and PRF membrane techniques.

Materials and Methods

Subject and Site selection
A total of 10 systemically healthy patients (males = 6; females = 4), visiting the outpatient section of the Periodontology dept. in having 20 Miller’s Class I/II isolated recession defects on the labial/buccal surfaces of maxillary anteriors and/or premolars were enrolled in the study. The study was approved by the Scientific and Ethics committee of the institution. Informed consent was obtained from all the participants along with a complete hemogram. This study was registered in the clinical trials registry under ClinicalTrials.gov with Reference No. NCT02835430.
Eligibility criteria
Age range between 18 – 45 years with bilateral Miller’s Class I/II isolated recession defects associated with maxillary anteriors and/or premolars and presence of adequate keratinized tissue were included in the study. Participants who were periodontally and systemically healthy and who could maintain good oral hygiene were included. History of prolonged use of medication; pregnancy/Lactating women; tobacco in any form; malaligned teeth; cervical abrasion defects; previous history of periodontal surgery on the selected areas; molar teeth were excluded.

Study design
Randomized prospective, double-blind, controlled, split-mouth design comparing the clinical efficacy of CAF and PRF with or without DFDBA included cause-related therapy and clinical measurements, surgical procedure, post-surgical instructions and re-evaluation at 6 months post-surgery. A total of 10 patients with bilateral isolated gingival recession defects, that is, 20 sites, were enrolled in the study.

Randomization
Each patient with bilateral isolated recession defect in the maxillary anteriors and/or premolars, participated in the study. The control sites and test sites were randomly assigned by a flip coin technique. The treatment for control sites was by CAF and PRF membrane technique and the test sites were treated by CAF and PRF membrane with DFDBA. Each patient underwent oral prophylaxis and received oral hygiene instructions. Plaster models were prepared from alginate impressions of the maxillary arch. Acrylic stents were fabricated for the selected sites. A groove was placed on the stent in line of recession which acted as a constant reference guide for standardized measurements. At baseline and 6 months follow-up postoperatively, the clinical parameters measured for both the sites were as follows:

i. Gingival index (GI) [Given by Loe and Silness in 1963]
ii. Plaque index (PI) [Given by Silness and Loe in 1964]
iii. Probing depth (PD):- Measured from the crest of gingival margin to the base of gingival sulcus.
iv. Recession depth (RD):- Recorded from the CEJ to the most apical extension of gingival margin.
v. Width of Keratinized Gingiva (WKG):- Measured from the gingival margin to the mucogingival junction.
vi. Clinical Attachment Level (CAL):- Measured from the CEJ to the base of the sulcus. At 6 months post-operatively, the root coverage percentage was also obtained based on the following formula:

\[
\frac{\text{Preoperative recession depth} - \text{Postoperative recession depth}}{\text{Preoperative recession depth}} \times 100
\]

The clinical measurements were recorded from the mid-labial aspect of the selected teeth by using a UNC-15 graduated periodontal probe and rounded to the nearest 0.5 mm.

**Surgical technique**

The surgical procedure for all the involved teeth was performed by one surgeon (P.D.). The control sites were treated using CAF and PRF. The test sites were treated using CAF and PRF with DFDBA. After administration of local anesthesia, root surface debridement was performed using Gracey curettes for the detoxification of the exposed root surface. A sulcular incision was given using a no. 15c surgical blade on the labial aspect of the selected tooth. From that point, split-thickness horizontal incisions were given on mesial and distal papillae, perpendicular to CEJ, not extending beyond 1 mm of the teeth next to the surgical site and becoming full-thickness beyond CEJ. For flap mobilization, 2 obliquely releasing incisions were given from the mesial and distal extremities of the horizontal incision to the alveolar mucosa and a split-full-split thickness flap was raised. Thorough debridement of the exposed area of the root surface was done to reduce the root convexities if any. Periosteal releasing incisions in line with the vestibular lining mucosa were performed to reduce the tension of residual muscle attachment, thereby, facilitating the coronal advancement and passive adaptation of the flap. De-epithelialization of anatomic papillae was done to create a bleeding bed.

**Preparation of PRF**

Intraoperatively, a 10 ml of blood sample of the patient was collected and centrifuged immediately at 3000 rpm for 10 minutes. The PRF clot was separated from the other two layers - acellular plasma and red blood cells. The PRF membrane was prepared in the PRF Box by squeezing out fluids from the fibrin clot. After pre-suturing at the control site, the membrane was placed on the exposed area of the root, exceeding the margins of the surrounding bone. At the test site, following pre-suturing, DFDBA was evenly layered to around 1 mm thickness for covering the exposed root and the surrounding bone. DFDBA was subsequently covered by PRF.
In both the groups, the membrane was stabilized by interrupted sutures using 4-0 Vicryl suture, and the flap was coronally displaced and sutured without tension by sling suturing using 4-0 Mersilk suture. Interrupted sutures were given at the releasing incisions followed by a periodontal dressing.

**Post-surgical instructions**
Post-surgical instructions were explained to the patients immediately after surgery. Mechanical cleaning refrained at the surgical site and use of 0.2 % chlorhexidine solution was recommended twice daily for 30 seconds for 14 days. Analgesics and antibiotics were prescribed. Suture removal was done 14 days post-surgery and all patients were demonstrated about the mechanical plaque control at the surgical site with the help of a soft-bristled toothbrush. The final examination was done 6 months post-operatively.

**Statistical analysis**
The data was entered and analyzed using the Statistical Package for Social Sciences (SPSS) for Windows 26.0. (SPSS, Inc. Chicago, Illinois) Confidence intervals were set at 95%, and a p-value ≤ of 0.05 was considered statistically significant. Descriptive statistics were expressed as mean ± standard deviation (SD) for all the parameters at different time intervals. The difference between each pair of measurements from baseline to 6 months was calculated. Wilcoxon Signed Ranks Test was used for the comparison between the groups from baseline to 6 months.

**Results**
Clinical parameters in both control and test groups at baseline and 6 months showed statistically significant (p<0.05) improvement in probing depth, recession depth, clinical attachment level and increase in the width of keratinized gingiva. Clinical parameters between the CAF+PRF group and CAF+PRF+DFDBA group were compared at 6 months post-surgery and there was no statistically significant difference. (p>0.05) The percentage of root coverage at 6 months post-surgery was statistically significant (p<0.05) in both control and test groups. At 6 months post-surgery, although the root coverage was found to be better with the addition of DFDBA, there was no statistically significant difference between both groups. (p>0.05)

**Discussion**
The results obtained from this study indicated that CAF and PRF membrane with or without DFDBA were effective in reducing the recession depth. This successful clinical outcome was maintained over 6 months and corresponded to the overall percentage RC of 79 ± 15.5% and 86 ± 15.4% for control and test groups, respectively. These findings correlate well with the previous studies where the root coverage ranged from 74% to 90%. There was a clinically significant increase in WKG and a reduction in the mean PD. Hence, the mean gain in CAL seemed to be retained during the follow-up period for both groups. Clinical improvement in the present test group was probably based on true periodontal regeneration. Conversely, the improvement in the control group of the current study was probably based on the formation of a long junctional epithelium/regenerative attempt. The use of DFDBA seemed to maintain the space necessary for periodontal regeneration, promoting true periodontal regeneration. The WKG gain in the control group could be due to the cicatricial properties of PRF, which contains several growth factors influencing the tissue manifestation proliferation of gingival or periodontal fibroblasts. It can also be correlated to the fact that the mucogingival junction tends to be located at its genetically determined position. Also, the granulation tissue derived from the periodontal ligament forms a connective tissue with the potential to induce epithelial keratinization.

The present study indicated that both treatment aspects could be successfully used for the management of Miller’s Class I and Class II isolated recession defects. Both the groups significantly demonstrated an overall improvement in the assessed clinical parameters. Although the result of this study found no significant difference between the two groups with regards to the treatment outcome, a trend towards greater improvement in recession reduction, clinical attachment level, and root coverage percentage was seen in the test group. The improved treatment outcomes in the test group could be attributed to the formation of new connective tissue attachment. However, periodontal regeneration can only be evaluated at the histologic level, which was not performed in the present study. The use of PRF membrane provided a fibrin matrix which was well-suited for manipulation and suturing of the CAF at the operated site. In the present study, the mean reduction in probing depth and gain in attachment level was evident, maybe due to periodontal regeneration which further requires histological confirmation.

When compared to FGG and SCTG, which require the creation of a second surgical site, the root coverage procedure based on CAF + PRF membrane + DFDBA significantly decreases the
surgical risk by eliminating the need for donor site along with reduced morbidity and no post-surgical bleeding.

**Conclusion**

Both the control and test group sites healed uneventfully. However, the above findings are suggestive of no additional benefit of using DFDBA. The histological evaluation regarding the nature of periodontal healing at the end of 6 months would have been the most appropriate method to evaluate the intergroup comparison outcomes in this study.

**References**

### Table 1: Clinical parameters of treated sites at baseline and 6 months

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Control Group</th>
<th>Test Group</th>
<th>Difference scores between both groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Mean ± SD)</td>
<td></td>
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<tr>
<td>Recession Depth</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>3.6 ± 0.84</td>
<td>3.7 ± 0.67</td>
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<tr>
<td>6 months</td>
<td>0.8 ± 0.63</td>
<td>0.6 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>2.8 ± 0.63*</td>
<td>3.1 ± 0.32*</td>
<td>0.3 ± 0.32</td>
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<tr>
<td>Probing Depth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.6 ± 0.52</td>
<td>1.9 ± 0.88</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.1 ± 0.32</td>
<td>1.2 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>0.5 ± 0.53*</td>
<td>0.7 ± 0.67*</td>
<td>0.2 ± 0.14</td>
</tr>
<tr>
<td>Clinical Attachment Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.2 ± 0.63</td>
<td>5.6 ± 1.17</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.9 ± 0.57</td>
<td>1.8 ± 0.79</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>3.3 ± 0.67*</td>
<td>3.8 ± 0.63*</td>
<td>0.5 ± 0.63</td>
</tr>
<tr>
<td>Width of Keratinized gingiva</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.6 ± 0.80</td>
<td>2.8 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>3.7 ± 0.90</td>
<td>3.9 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>1.1 ± 0.32</td>
<td>1.1 ± 0.32</td>
<td>1.1 ± 0.32</td>
</tr>
<tr>
<td>Percentage Root Coverage (%)</td>
<td>79 ± 15.5*</td>
<td>86 ± 15.4*</td>
<td>7 ± 4.95</td>
</tr>
</tbody>
</table>

Wilcoxon Signed Rank Test, *significant at p<0.05, SD – Standard Deviation

### Table 2: Clinical Indices of treated sites at baseline and 6 months

<table>
<thead>
<tr>
<th></th>
<th>Gingival Index (Mean ± SD)</th>
<th>Plaque Index (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Test group</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.72 ± 0.32</td>
<td>0.82 ± 0.16</td>
</tr>
<tr>
<td>6 months</td>
<td>0.4 ± 0.29</td>
<td>0.35 ± 0.17</td>
</tr>
<tr>
<td>Difference</td>
<td>0.32 ± 0.16</td>
<td>0.47 ± 0.18</td>
</tr>
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</table>

SD – Standard Deviation