## Original Article

# Comparative evaluation of human chorion membrane and platelet-rich fibrin membrane with coronally advanced flap in treatment of Miller's class I and II recession defects: A randomized controlled study

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**Abstract:**

**Background:** Root coverage procedures have gained much popularity in recent years, due to increasing esthetic demands of patients. Coronally advanced flap (CAF) is a predictable technique for treating gingival recessions. Platelet-rich fibrin (PRF), an autologous platelet concentrate, has properties to enhance soft-tissue wound healing. A current method in dentistry is the use of human chorion membrane, a placental derivative, having biologic properties that help in healing and regeneration. Thus, the aim of the study was evaluation and comparison of the efficacy of chorion membrane and PRF membrane in the treatment of Miller's Class I and Class II recession defects. **Materials and Methods:** This was a randomized controlled clinical study. Totally 30 sites with Miller's Class I and Class II recession were taken and randomly allocated to chorion membrane (test) PRF membrane (control) group. The clinical parameters recorded were clinical attachment level (CAL), recession height (REC-HT), recession width (REC-WD), width of keratinized gingiva (WKG) and gingival tissue thickness (GTH). **Results:** Significant differences were seen from baseline to 6 months in test group regarding gain in CAL ($P < 0.001$), reduction in REC-HT ($P < 0.001$), decrease in REC-WD ($P = 0.02$), increase in WKG ($P < 0.001$), and increase in GTH ($P < 0.001$). In the control group also, significant difference was noted at the end of 6 months i regarding gain in CAL ($P < 0.001$), reduction in REC-HT ($P < 0.001$), decrease in REC-WD ($P = 0.029$), increase in WKG ($P < 0.001$), and increase in GTH ($P < 0.001$). Intergroup analysis showed significant differences between test and control groups at the end of 6 months, with CAL, REC-HT, WKG, and GTH showing statistically significant differences with $P = 0.002$, $0.001$, $0.001$, and $0.026$, respectively. No significant difference was seen regarding REC-WD ($P = 0.39$). **Conclusions:** Both are effective materials in root coverage, but chorion membrane showed better and more stable results at the end of 6 months as compared to PRF membrane in treating gingival recession.

**Key words:**
Coronally advanced flap, gingival recession, human chorion membrane, platelet-rich fibrin

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## INTRODUCTION

An optimum association between teeth, neighboring oral tissues, and the periodontal complex results in a perfect smile. Gingival recession is the "apical migration of the gingival margin below the cemento-enamel junction (CEJ)."[1] It is perceived by the patients as increase in the length of teeth. It can also be associated with other factors, such as tooth hypersensitivity, pain, and carious and noncarious lesions. Gingival recession is a multifactorial disease commonly caused by the accumulation of plaque biofilm resulting in inflammatory periodontal diseases and due to trauma caused by faulty and aggressive tooth brushing. Other risk factors include aging, dehiscence of alveolar bone, high frenum attachment, smoking, tooth malposition, and

iatrogenic factors associated with various periodontal and restorative procedures.[2]

The coronally advanced flap (CAF) is a commonly used method for root coverage, and it has demonstrated very good results in treating gingival recessions regarding root coverage and esthetic outcomes.[3]

Ideal aim of periodontal treatment should be the restoration of form and function of lost structures. First described by Nyman *et al.*,[4] in 1982, guided tissue regeneration (GTR) procedures initially employed nonbiodegradable (nonabsorbable) barriers that were surgically removed from the wound site after a period of healing. More recently, numerous absorbable barrier membranes have been introduced that appear to offer certain advantages over traditional nonabsorbable membranes. According to Melcher's hypothesis,[5] there are certain cells present in the periodontium, which have the potential of periodontal regeneration, when the opportunity is provided to them to populate the periodontal wound. A study was done by Lee *et al.*,[6] concluded that the connective tissue attachment that is solely obtained by CAF is not stable for a long period, and various adjuncts have been employed to improve clinical outcomes as well as healing.

### Platelet-rich fibrin and chorion membrane

Platelet-rich fibrin (PRF) is a platelet concentrate (second generation). It was initially developed by Choukroun *et al.*, 2001 in France.[7] It can be used straight away as a clot or as a strong membrane after compression. PRF can act as an adjunct to the natural healing process and can provide mechanical support and protection to graft material, release various growth factors- platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, insulin-like growth factor 1, and vascular endothelial growth factor (VEGF), thus facilitating healing, self-regulation of inflammatory process mediated through leukocytes and various cytokines present within it, and the fibrin network helps in cellular migration of endothelial cells.[8] Hence, we can expect less postsurgical discomfort, more rapid soft-tissue healing with less edema compared to subepithelial connective tissue graft, and a relatively unlimited source of graft material. In addition, the need for a donor site is eliminated, making this technique less invasive.

Placental allografts have been used successfully for a variety of applications since the last 70 years. They possess antimicrobial and antibacterial properties and have shown to reduce inflammation and provide a protein-rich matrix, thereby facilitating the migration of cells at the defect area.[9] The extracellular matrix components include Collagen (types I, III, IV, V, and VI), proteoglycans, fibronectin, and laminin. Chorion membrane has shown to reduce scar formation and inflammation, enhancing healing of the wound. It acts as a scaffold for proliferation and differentiation of cells due to its antimicrobial properties. The extracellular matrix and its components, such as growth factors, make it an excellent biomaterial to act as a native scaffold for tissue engineering. It is easy to procure and process. Its matrix contains growth factors such as keratinocyte growth factor, basic fibroblast growth factor, and transforming growth factor-b, which help in regenerating the periodontium[10] and give a setting for faster healing.[11]

The hypothesis tested in this study was that there would be no difference between the clinical effectiveness of human chorion membrane and PRF membrane in the treatment of Miller's Class I and Class II recession defects.

## MATERIALS AND METHODS

### Patient characteristics

Twenty adult patients, 20–59 years of age (all nonsmokers), were recruited in this study. The study was carried out on 30 sites with Miller's Class I or Class II recession defects. The sites were divided into two groups. The test and control groups consisted of 15 sites each. The research protocol for this randomized controlled clinical study was approved by the Institutional Ethics Committee, and all participants screened for the study signed informed consent statements after discussion regarding any questions they had with the study investigator(s).

The criteria for selection were systemically healthy participants between the age group of 18–60 years, maxillary and mandibular incisors, canines and premolars having Miller's Class I or II gingival recession, and participants maintaining good oral hygiene after completion of scaling and root planing. Exclusion criteria were pregnant women or lactating mothers, participants who had undergone any mucogingival procedures at the selected sites within the previous 3 months, allergy to local anesthesia and chlorhexidine (CHX), antibiotic, and analgesic, which would be prescribed and individuals having habit of smoking and tobacco chewing. The primary investigator was blinded to the allocation and only measured the outcomes whereas all the surgical procedures were carried out by another operator. Hence, it was a single-blind study.

### Presurgical phase

Initially, scaling and root planing of all quadrants were done using hand and ultrasonic instruments and oral hygiene instructions were given, selective occlusal grinding was done if indicated. Recording of baseline parameters was done after initial preparation was completed, within 14 days before surgery (or on the day of surgery). The initial baseline examination assessed recession height (REC-HT), recession width (REC-WD), clinical attachment level (CAL), width of keratinized gingiva (WKG), and gingival tissue thickness (GTH). This was a parallel design study in which the recession defects were randomly assigned by a computer-generated table to receive either chorion membrane (test) or PRF membrane (control) group, and the results of the randomization were sealed in an opaque envelope for allocation. In this study, 20 patients with a total of 30 defects completed the study. Before surgery, plaque index was recorded to assess the maintenance of oral hygiene by the patient. An acrylic stent was made for every patient and stored on the study cast to minimize distortion. The stent was grooved in an occlusoapical direction with a tapered bur so that the standard periodontal probe goes into the same position for each successive measurement.

### Measurements

All baseline clinical parameters were recorded no more than 14 days prior to or on the day of surgery. A calibrated probe was used to make measurements and recorded to the nearest

millimeter. REC-HT was measured from CEJ to free gingival margin, [Figure 1] REC-WD was measured mesiodistally at the CEJ, CAL was measured from the CEJ to the base of the pocket, WKG was measured from the most apical point on free gingival margin to mucogingival junction, and GTH was measured at midpoint location between the most apical point of gingival margin and mucogingival junction using image analyzer with stereomicroscope. After topical anesthesia, a 1½" needle with a rubber stopper was pierced perpendicular to the mucosal surface, through the soft tissue, until hard surface was felt. The rubber stopper had marked this level. The distance between the needle tip and the rubber stopper was then measured under a stereomicroscope using image analyzer. These measurements were evaluated at baseline and at 6 months interval.

### Surgical procedures
Following measurement recordings and administration of LA, horizontal incisions were given at base of interdental papilla, on either side of involved tooth, without involving gingival margin of adjacent tooth. Then, two vertical incisions were given, starting from the mesial and distal extremities, extending apically from the horizontal incisions, one from each side and extending beyond the mucogingival junction [Figure 2]. Full-thickness mucoperiosteal flap was raised till the mucogingival junction, and beyond mucogingival junction, a partial thickness flap was raised to make the flap mobile [Figure 3]. The papillae adjacent to the involved tooth were deepithelialized. The exposed root was debrided with hand and ultrasonic instruments. Apical to bone exposure, flap elevation continued split thickness and finished when it was possible to move the flap passively in the coronal direction. To permit the coronal advancement of the flap, all muscle insertions present in the thickness of the flap were eliminated.

For control group, PRF was prepared. PRF clot is immediately converted into membrane. Irradiated, sterilized, and processed human chorion membrane was procured and cut into appropriate size according to the size of the recession defect and then placed into the defect in the test group [Figure 4]. The flap was sutured using 5-0 polyglycolic acid sutures followed by a non-eugenol pack [Figure 5].

### Postsurgical phase
All patients were recalled after 7 days for pack removal. Patients were given antibiotics and analgesics for 5 days and were instructed not to brush the teeth in the treated area but to rinse with CHX solution (0.2%) twice daily for 1 min. Patients were called after 3 months for supragingival debridement and then at 6 months for follow-up measurements of CAL, REC-HT, REC-WD, WKG, and GTH.

### Statistical analysis
Sample size calculation was done based on the formula, $n = 2 \times (z \times SD/d)^2$ (95% confidence interval and 80% power). The analysis was done to compare the changes in clinical parameters from baseline measurements. The statistical tests used were Paired *t*-test to compare baseline data with 6 months data in both test and control groups. Independent *t*-test was performed to compare clinical parameters between the two groups at baseline and 6 months postoperatively. Differences were considered to be statistically significant at $P < 0.05$.

## RESULTS

All 20 patients completed the treatment, and no adverse reactions were experienced related to the treatment. Postsurgical healing was uneventful in all the 30 sites involved in the study.

The mean age of the test group was 38.67 years, and the control group was 33.60 years. There was no statistical difference between the two groups with respect to age ($P = 0.22$). The number of males in the test group was 10 and in the control group were 13 whereas the number of females in the test group was 5 and in the control group was 2.

In the test group (chorion membrane), from baseline to 6 months period, statistically significant differences were seen regarding gain in CAL from 5.53 ± 1.12 to 2.60 ± 1.05 ($P < 0.001$), reduction in REC-HT from 4.0 ± 0.75 to 0.67 ± 0.90 ($P < 0.001$), decrease in REC-WD from 4.13 ± 0.83 to 3.73 ± 1.03 ($P = 0.02$), increase in WKG from 5.87 ± 3.31 to 8.73 ± 3.45 ($P < 0.001$), and increase in GTH from 0.98 ± 0.14 to 1.25 ± 0.19 ($P < 0.001$ [Table 1].

In the control group (PRF membrane), from baseline to 6 months period, statistically significant differences were seen regarding gain in CAL from 4.73 ± 0.96 to 2.87 ± 0.91 ($P < 0.001$), reduction in REC-HT from 2.87 ± 0.74 to 0.73 ± 0.70 ($P < 0.001$), decrease in REC-WD from 3.0 ± 0.92 to 2.53 ± 0.91 ($P = 0.029$), increase in WKG from 5.73 ± 2.76 to 7.67 ± 2.89 ($P < 0.001$), and increase in GTH from 0.98 ± 0.17 to 1.16 ± 0.22 ($P < 0.001$) [Table 2].

The clinical parameters of both the test and control groups were compared at the end of 6 months. Table 3 shows the difference of clinical parameters between test and control group at the end of 6 months period [Figure 6]. When the differences between baseline and follow-up in clinical parameters were compared (intergroup), CAL, REC-HT, WKG, and GTH showed statistically significant differences with $P = 0.002$, 0.001, 0.001, and 0.026, respectively. No significant difference was seen regarding REC-WD ($P = 0.39$) [Table 3].

### Table 1: Clinical parameters at baseline and 6 months in test group (intragroup analysis) (mean±standard deviation)

| | Mean±SD | | | | |
| --- | --- | --- | --- | --- | --- |
| | CAL | REC-HT | REC-WD | WKG | GTH |
| Baseline | 5.53±1.12 | 4.0±0.75 | 4.13±0.83 | 5.87±3.31 | 0.98±0.14 |
| 6 months | 2.60±1.05 | 0.67±0.90 | 3.73±1.03 | 8.73±3.45 | 1.25±0.19 |
| Difference (baseline-6 months) | 2.93±1.03 | 3.33±1.04 | 0.40±0.63 | −2.86±0.83 | −0.27±0.12 |
| *P* | <0.001** | <0.001** | 0.02* | <0.001** | <0.001** |

$P \leq 0.05$ considered statistically significant. *Statistically significant; **Statistically highly significant. CAL – Clinical attachment level; REC-HT – Recession height; REC-WD – Recession width; WKG – Width of keratinized gingiva; GTH – Gingival tissue thickness; SD – Standard deviation; *P* – *P* value

**Table 2: Clinical parameters at baseline and 6 months in control group (intragroup analysis) (mean±standard deviation)**

| | CAL | REC-HT | REC-WD | WKG | GTH |
|---|---|---|---|---|---|
| Baseline | 4.73±0.96 | 2.87±0.74 | 3.0±0.92 | 5.73±2.76 | 0.98±0.17 |
| 6 months | 2.87±0.91 | 0.73±0.70 | 2.53±0.91 | 7.67±2.89 | 1.16±0.22 |
| Difference (baseline-6 months) | 1.86±0.64 | 2.13±0.64 | 0.46±0.74 | −1.93±0.45 | −0.18±0.09 |
| $P$ | <0.001** | <0.001** | 0.029* | <0.001** | <0.001** |

$P \leq 0.05$ considered statistically significant. *$P \leq 0.05$ – Significant; **Statistically highly significant. CAL – Clinical attachment level; REC-HT – Recession height; REC-WD – Recession width; WKG – Width of keratinized gingiva; GTH – Gingival tissue thickness; $P$ – $P$ value

**Table 3: Difference of clinical parameters of test and control group at the end of 6 months (intergroup analysis)**

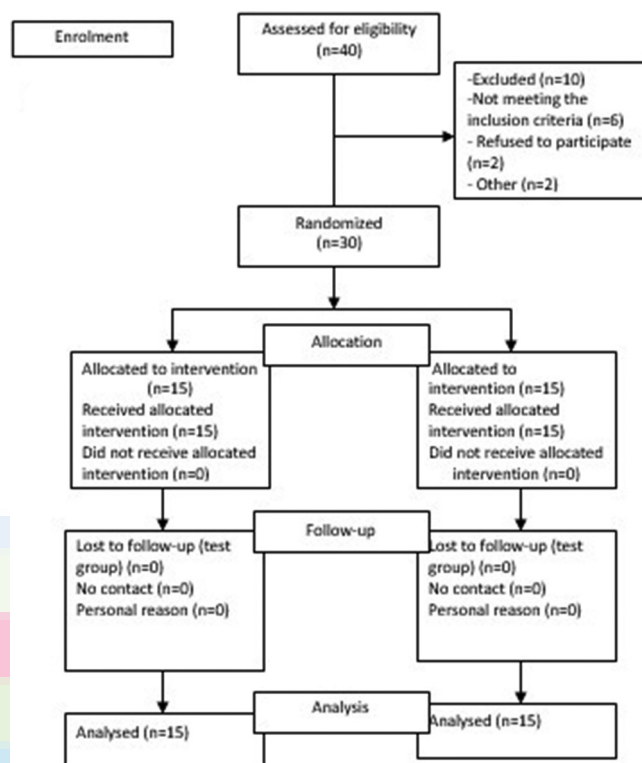| | Mean difference | $P$ | 95% CI of the difference | |
|---|---|---|---|---|
| | | | Upper | Lower |
| CAL | 1.067 | 0.002** | 0.42 | 1.70 |
| REC-HT | 1.20 | 0.001** | 0.55 | 1.84 |
| REC-WD | −0.20 | 0.39 | −0.67 | 0.27 |
| WKG | 0.93 | 0.001** | 0.43 | 1.43 |
| GTH | 0.09 | 0.026* | 0.01 | 0.17 |

$P \leq 0.05$ considered statistically significant. *Statistically significant; **Statistically highly significant. CAL – Clinical attachment level; REC-HT – Recession height; REC-WD – Recession width; WKG – Width of keratinized gingiva; GTH – Gingival tissue thickness; CI – Confidence interval; $P$ – $P$ value

## DISCUSSION

This study was planned to correct Class I and Class II recession defects using CAF along with either chorion or PRF membrane, after which the results of both the groups were evaluated and compared. Miller's Class I and Class II recession defects were chosen in the study as there is no interdental soft tissue or bone loss in such defects and 100% root coverage can be achieved.[12] In this randomized controlled clinical study, the population of patients chosen was well-balanced and carefully selected according to strict entry criteria and randomization process, and the procedures were conducted within a common and tight protocol [Flowchart 1].



Flowchart 1: Consort flowchart for the patient recruitment. *n* – number of participants

Mesenchymal stem cells are one of the most important cells which help in carrying out each phase of the wound healing process: Inflammatory phase, proliferative phase, and remodeling. Chorion is composed of three layers: Reticular layer, basement membrane, trophoblasts. Collagen (types I, III, IV, V, and VI), proteoglycans, fibronectin, and laminin are found in chorion.[11] Its matrix contains growth factors, such as keratinocyte growth factor, transforming growth factor-b, and basic fibroblast growth factor which help in regeneration of periodontium[10] and provide an habitat for faster healing.[11] Collagen is a bioabsorbable hemostatic material which stimulates the migration of adjoining connective tissue.[13] Fibronectin helps in repair of tissue, clotting of blood, cell migration, and adhesion.[13,14] Due to the presence of laminin, the chorion membrane is self-adhering and does not require suturing separately. Looking into the various advantages of chorion, it was decided to use the material in this study.

Choukroun's PRF is a second-generation platelet concentrate and has a clearly defined composition and three-dimensional structure.[7] It is easy to prepare, apply, minimally expensive, and does not require any biochemical modification. Main drawback of PRF is that storage after its preparation

is difficult.[15] In addition, membranes must be used immediately after preparation as they shrink, thus leading to dehydration, which will alter the biologic properties. Dehydration will also lead to a decrease in the content of growth factors in PRF. Hence, in our study, PRF was prepared immediately after starting the CAF surgery. The biological principles of healing in root coverage procedures include histocompatibility, vascularization, wound contraction, and wound asepsis.[16] The property of angiogenesis of PRF matrix is by the three-dimensional arrangement of the fibrin gel and by the effect of cytokines that are trapped in the mesh. The main angiogenesis factors, fibroblast growth factor-basic, VEGF, angiopoietin, and PDGF are present in the fibrin gel. Fibronectin, fibrin, TGF-β, and PDGF play an essential role for modulating integrin expression and proliferation and migration of fibroblasts in the wound. PRF can thus help in microvascularization and guide epithelial cell migration and hence accelerate healing.[7] Hence, in this study, PRF membrane was placed under the CAF to accelerate healing so that increased initial stability and root coverage can be obtained.

The results in test group showed significant improvements from baseline to 6 months. The CAL showed statistically significant

**Figure 1:** Recession height at test and control site at baseline



**Figure 2:** Incision of coronally advanced flap



**Figure 3:** Flap reflection and placement of chorion membrane at test site and platelet rich fibrin membrane at control site, respectively



**Figure 4:** Suturing



**Figure 5:** Periodontal pack



**Figure 6:** Recession coverage at the end of 6 months

improvement ($P < 0.001$). The result was similar to the results obtained by Esteves *et al*.[17] in which the CAL improved from $4.67 \pm 1.39$ to $1.19 \pm 0.87$ at the end of 6 months. In our study, REC-HT and REC-WD also showed significant differences from baseline to 6 months ($P < 0.001$ and $P = 0.02$, respectively). In a similar study was done by Pundir *et al*. (2015)[18] using chorion membrane, the REC-HT showed significant improvement from $3.16 \pm 0.86$ to $1.30 \pm 0.46$ at the end of 6 months.

The WKG showed significant improvements in a study done by Pundir *et al*. (2015)[18] comparing chorion and amnion membranes. It increased from $3.46 \pm 0.13$ to $4.53 \pm 0.17$ at the end of 6 months. Similar results were obtained in our study ($P < 0.001$). The GTH showed significant improvement from $0.98 \pm 0.14$ to $1.25 \pm 0.19$ at the end of 6 months ($P < 0.001$). Thus, the chorion membrane was successful in augmenting the thickness of the gingival tissue also. Assessing the periodontal biotype is important as it decides the recurrence of recession, hence, it was necessary to measure this parameter. The results of the control group also showed significant improvements from baseline to 6 months. There was improvement in CAL from $4.73 \pm 0.96$ to $2.87 \pm 0.91$ ($P < 0.001$). The results were similar to study done by Padma *et al*.[19] where they found CAL reduced from $4.75 \pm 1.29$ to $1.00 \pm 0.00$ at 6 months. REC-HT showed significant improvement from baseline to 6 months ($P < 0.001$) and REC-WD reduced from $3.0 \pm 0.92$ to $2.53 \pm 0.91$ ($P = 0.02$). There was a significant improvement in and GTH ($P < 0.001$) at the end of 6 months.

Further, the intergroup analysis was done to compare both test and control groups. This is the first study that has compared chorion membrane and PRF membrane for the treatment of

recession defects. The results showed significant differences regarding CAL ($P = 0.002$), REC-HT ($P = 0.001$), WKG ($P = 0.001$), and GTH ($P = 0.02$) between the two groups. The REC-WD did not show significant differences between the two groups ($P = 0.39$) at the end of 6 months.

Conventionally, the preparation of a PRF membrane is done by the compression of a PRF clot by moist or dry gauze. However, Su *et al*.[20] and Burnouf *et al*.[21] showed that considerable quantity of growth factors involved in the regeneration of tissues are eliminated by squeezing, and the compression has also shown to damage the platelets. This could be one of the reasons for an inferior result in the control group in comparison to the test group. A study was done by Aroca *et al*.[15] also reported an inferior root coverage with the membrane as compared to CAF alone in multiple recessions. The main factor that decides the favorable outcome of treatment with GTR is the physical property of the membrane that is being used, specifically the configuration and mechanical strength of the membrane. The chorion membrane, a placental derivative, has greater rigidity as compared to membrane prepared from PRF, a blood derivative. This property of the material might have led to the superior result obtained with the membrane.

There are a few limitations of this study. First, the percentage of root coverage was not evaluated in this study. In addition, a single PRF membrane was placed on the defect site. PRF can be considered as having a nonhomogenous matrix as it has platelet aggregates and leukocytes concentrated at different ends, it would have been better if two membranes would have been placed in opposite directions so as to have a uniform effect over the entire defects.

Overall, both the groups showed good results in reducing the recession, but chorion showed superior results compared to PRF membrane. In addition, inference can be drawn that there are materials that can be used instead of connective tissue graft, which although is considered as the gold standard, is associated with significant patient discomfort and increased operating time.

These results specify that both are efficacious materials in root coverage, but chorion membrane showed better results compared to PRF with respect to the clinical parameters, namely CAL, REC-HT, WKG, and GTH at the end of 6 months. Further studies need to be carried out with larger sample size and for a longer duration to further explore their roles as a membrane in the management of gingival recession.

## CONCLUSIONS

Chorion membrane proved to be a significantly better material as compared to PRF membrane in this 6 month randomized controlled study for treatment of gingival recession defects.

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### Conflicts of interest

There are no conflicts of interest.

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