

Chorion Membrane and Concentrated Growth Factor Membrane in Treating Bilateral Miller's Class I and II Gingival Recessions in Maxillary Premolars – a Clinical Study

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ABSTRACT

Background: Periodontitis causes many clinical presentations, one of which is gingival recession, which leads to root exposure, hypersensitivity and esthetic issues. One of the most accepted surgical procedures of root coverage is the coronally advanced flap (CAF) technique. Newer regenerative modalities such as placental-derived chorion membranes and autologous concentrated growth factor (CGF) membranes have a higher capacity to heal because of the bioactive nature of these membranes.

Materials and methods: A randomized controlled clinical trial study was carried out on 10 patients with bilateral gingival recession in maxillary premolars. They happened to receive CAF with chorion membrane on one side (Group A) and CGF membrane on the other side (Group B). Such clinical parameters as probing depth (PD), clinical attachment level (CAL), recession height (RH), recession width (RW) and width of keratinized tissue (WKT) were measured at baseline and after three months of follow-up. Paired and unpaired t-tests were undertaken through IBM SPSS 23.0.

Results: In both groups, all clinical parameters improved statistically significant after the surgery ($p < 0.05$). Group A root coverage mean was 89 percent and Group B 82 percent. The postoperative values

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of PD, CAL, RH, RW and WKT did not differ statistically between the two groups ($p > 0.05$), but Group A had slightly superior results.

Conclusion: Human chorion membrane and concentrated growth factor membrane being used with CAF lead to effective root coverage and clinical improvement, which is considered significant in Miller Class I and II gingival recessions. Both membranes are effective, but the chorion membrane might provide slightly better clinical results. Longitudinal research involving bigger samples should be done.

Keywords: gingival recession, coronally advanced flap, chorion membrane, concentrated growth factor, root coverage, periodontal regeneration, clinical attachment level.

INTRODUCTION

Inflammation of the periodontal supporting tissue is termed as periodontitis, which leads to the destruction of both hard and soft tissues around the tooth. The major criteria of periodontitis are tooth mobility, alveolar bone loss and clinical attachment loss, including recession of gingiva and increase in probing depth. Gingival recession is nothing but apical migration of the gingival margin that leads to the exposure of the root surface or unaesthetic elongation of the root portion. Gingival recession is classified into four classes, namely Class I, Class II, Class III and Class IV, by Miller (1).

Gingival recession results in plaque and calculus accumulation, tooth abrasion, root caries, dentinal hypersensitivity due to exposure of root dentin and, most importantly, unesthetic appearance causing discomfort to patients. Therefore, the management of gingival recession is a compulsory procedure to prevent loss of function and to manage the esthetic concern of patients. Though various techniques have been proposed for managing gingival recessions, the coronally advanced flap technique (Norberg, 1926) (2) is the mostly used procedure in patients with adequate amount of keratinized gingiva. Also, recession can be treated with various regeneration modalities, which include the use of guided tissue regeneration membranes with stem cells.

Placental membranes have anti-inflammatory and anti-microbial properties (3). Among them is the chorion membrane that contains growth factors and biological proteins required for regeneration. Growth factors are polypeptide molecules that enhance healing by modulating the cell responses such as cell attachment, adhesion, survival, proliferation, chemotaxis and differentiation (4). In 2006, Sacco developed the con-

centrated growth factor (CGF), a fibrin rich matrix containing growth factors and cellular components (platelets, leukocytes, CD34+ stem cells) that enhance the regeneration process (5).

The CGF can be pressed to form barrier membranes which can be used in guided tissue regeneration techniques. The main aim of this study is to compare chorion membrane and CGF membrane in treating Miller's Class I and II gingival recession in maxillary premolars.

MATERIALS AND METHODS

In order to compare the root coverage procedure with chorion membrane and CGF membrane, we carried out a three-month comparative controlled clinical trial on the treatment of bilateral Miller's Class I and II gingival recession of maxillary premolars. Ten patients with bilateral gingival recession involving maxillary premolars were selected. Using the coin toss method, the area to be treated was divided into two groups, A and B. The sites of Group A were treated with coronally advanced flap and chorion membrane, and those of Group B with coronally advanced flap and CGF membrane. The clinical parameters of both sites were examined at baseline (preoperatively) and at three months (postoperatively). Informed consent was obtained from the participating subjects before the commencement of the study. The study protocol was revised and approved by the institutional review committee.

Study design

We conducted a randomized controlled clinical trial on subjects selected from the outpatient department, Department of Periodontics, Adhiparasakthi Dental College and Hospital, Melmaruvathur, who came with the complaint of gingival

recession or dentinal hypersensitivity due to root exposure.

Eligibility criteria

Inclusion criteria comprised adults of either gender (18-40 years), Miller's Class I or II gingival recession > 2 mm in maxillary premolars and vital teeth free from dental caries.

Patients with smoking habit, poor oral hygiene maintenance, those with systemic illness, pregnant women and lactating mothers were all excluded.

Initial therapy

Every patient had to undergo initial phase I therapy, which included patient motivation, diet counselling, complete scaling and root planning. Hematological investigations were done prior to phase I therapy.

Stent preparation

After initial therapy, an acrylic stent was made using denture base resins with notch aiding for the placement of the probe during measurement. To prevent polymerization shrinkage, the stents were placed back in their respective casts after baseline evaluation.

Clinical parameters

The clinical parameters, including clinical probing depth, clinical attachment level, width of keratinized tissue, recession height and width, were recorded using the University of North Carolina (UNC-15) probe for each surgical site before surgery (baseline) and at three months after surgery.

Concentrated growth factor (CGF) preparation

For the preparation of CGF, intravenous blood from each patient was collected in 10-mL glass-coated plastic tubes without the addition of anti-coagulant solutions and it was immediately centrifuged by a CGF machine (Medifuge, Silfradent, Sofia, Italy) using a program with the characteristics of 30" acceleration, 2' 2700 rotations per minute (r.p.m)/735 g, 4' 2400 r.p.m/580 g, 4' 2700 r.p.m/735 g, 3' 3000 r.p.m/905 g and 36" deceleration and stop, as shown in Figure 1A, 1B.

After the program, four blood fractions were obtained. The superior phase contained the serum, the interim phase – the fibrin buffy coat,

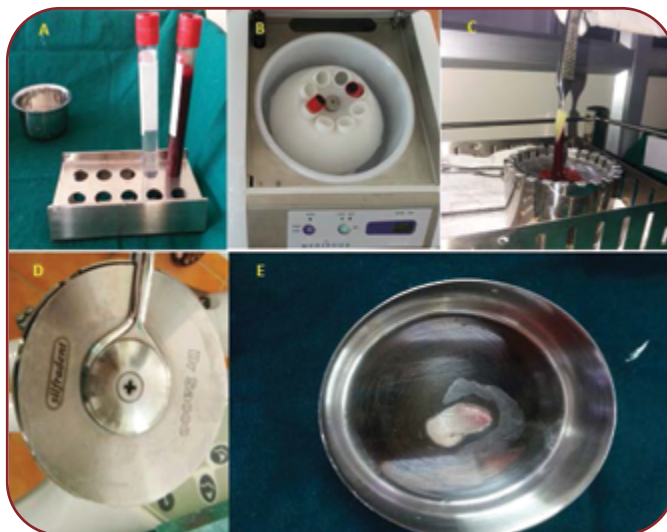


FIGURE 1. Concentrated growth factor membrane preparation. **A.** Blood collected for centrifugation; **B.** Centrifuge, Medifuge, Silfradent; **C.** Concentrated growth factor obtained after centrifugation; **D.** Preparation of CGF membrane using membrane plier; and **E.** Prepared concentrated growth factor membrane.

the third layer had growth factor concentrates and the fourth layer – red blood cells. The CGF clot was removed using sterile tweezer and the solid CGF was obtained. The CGF was compressed using a membrane plier to obtain a CGF membrane, which was immediately placed over the surgical area, as shown in Figure 1C, 1D, 1E.

Surgical technique (Group A)

Povidone iodine solution was used to isolate the surgical site. After adequate local anaesthesia with 2% lignocaine HCL containing 1:80,000 adrenaline, crevicular incisions were placed using No:15 Bard Parker Blade. De-epithelialization on the interdental papilla was done to create a connective tissue bed and a full thickness mucoperiosteal flap was reflected as shown in Figure 2F.

Curettes were used to plane the exposed root surface and then chorion membrane (freeze-dried, irradiated chorion), obtained from Tata Memorial Hospital Tissue Bank of Mumbai, India, was placed over the root surfaces, as illustrated in Figure 2G, and the flap was coronally placed and sutured with 3-0 (polyglycolide-colactide) sutures, as shown in Figure 2H. After hemostasis and clot stabilization were achieved, the periodontal dressing was placed over the surgical area, as shown in Figure 2I.



FIGURE 2. Surgical technique carried out in Group A patients. **A.** Preoperative clinical view; **B.** Probing depth and clinical attachment level measurement; **C.** Recession height measurement; **D.** Recession width measurement; **E.** Width of keratinized tissue measurement; **F.** Flap reflection; **G.** Placement of the chorion membrane; **H.** Coronal advancement of flap and suturing; and **I.** Placement of periodontal dressing



FIGURE 3. Surgical technique carried out in Group B. **A.** Preoperative clinical view; **B.** Probing pocket depth and clinical attachment level measurement; **C.** Recession height measurement; **D.** Recession width measurement; **E.** Width of keratinized tissue measurement; **F.** Flap reflection; **G.** Placement of concentrated growth factor membrane; **H.** Coronal advancement of flap and suturing; and **I.** Placement of periodontal dressing



FIGURE 4. Postoperative clinical view in Group A. **A.** Postoperative view at three months after surgery; **B.** Postoperative probing depth measurement; and **C.** Postoperative width of keratinized tissue measurement



FIGURE 5. Postoperative clinical view in Group B. **A.** Postoperative view at three months after surgery; **B.** Postoperative probing depth measurement; and **C.** Postoperative width of keratinized tissue measurement

Surgical technique (Group B)

Povidone iodine solution was used to isolate the surgical site. After adequate local anaesthesia with 2% lignocaine HCL containing 1:80,000 adrenaline, crevicular incisions were placed using No:15 Bard Parker Blade. De-epithelialization on the interdental papilla was done to create a connective tissue bed and a full thickness

mucoperiosteal flap was reflected as shown in Figure 3F. Curettes were used to plane the exposed root surface and then the CGF membrane was placed over the root surfaces and the flap was coronally placed and sutured with 3-0 (polyglycolide-colactide) sutures, as illustrated in Figure 3G and 3H. Hemostasis and clot stabilization were achieved and then periodontal dressing

sing was placed over the surgical area, as shown in Figure 3I.

Postoperative care

Antibiotics and analgesics were given for pain and swelling every eight hours as needed. Patients were instructed not to eat solid food or brush their teeth at the treated site but to rinse with chlorhexidine digluconate (0.2%, CHX) two times a day. Periodontal dressing and sutures were removed 10 days after surgery. Patients were kept under observation and were recalled every month postoperatively to assess their oral hygiene status. Clinical parameters were measured at three months after surgery.

Statistical analysis

IBM SPSS 23.0 software was used for data collection. The data obtained by us were analyzed to calculate the mean and standard deviation (SD) for probing pocket depth (PPD), clinical attachment level (CAL), recession height, recession width (RW) and width of the keratinized tissue (WKT). We used student's unpaired t-test for intergroup comparisons and student's paired t-test for intragroup comparisons. □

RESULTS

The clinical parameters were measured before surgery (Figure 2B, 2C, 2D for Group A, and Figure 3B, 3C, 3D for Group B) and three months postoperatively (Figure 4B, 4C for Group A and Figure 5B, 5C for Group B).

Comparison of mean baseline values between Groups A and B

The mean probing depth was 1.20 ± 0.13 mm for Group A and 1.20 ± 0.13 mm for Group B, with a p value of 1.0000. The mean CAL was 4.00 ± 0.21 mm for Group A and 4.00 ± 0.25 mm for Group B, with the p value of 1.0000. The mean RH was 2.80 ± 0.24 mm for Group A and 2.80 ± 0.20 mm for Group B, with the p value of 1.0000. The mean RW was 2.50 ± 0.22 mm for Group A and 3.00 ± 0.21 mm for Group B, with the p value of 0.1211. The mean WKT was 4.40 ± 0.27 mm for Group A and 4.30 ± 0.34 mm for Group B, with the p value of 0.8180. On comparing baseline values between Groups A and B, the difference was statistically non-significant ($P < 0.05$). Data are listed in Table 1.

TABLE 1. Comparison of mean baseline values between Groups A and B

Variables	Group A			Group B			t test	P value
	N	Mean (mm)	±SE	N	Mean (mm)	±SE		
PD	10	1.20	0.1333	10	1.20	0.1333	0.00	1.0000
CAL	10	4.00	0.2108	10	4.00	0.2582	0.00	1.0000
RH	10	2.80	0.2494	10	2.80	0.2000	0.00	1.0000
RW	10	2.50	0.2236	10	3.00	0.2108	1.63	0.1211
WKT	10	4.40	0.2667	10	4.30	0.3350	0.23	0.8180

N=number; SE=standard deviation error; mm=millimetre; PD=probing depth; CAL=clinical attachment level; RH=recession height; RW=recession width; WKT=width of keratinized tissue.

*P value less than 0.05 is considered statistically significant.

TABLE 2. Comparison of mean baseline and postoperative values of clinical parameters in Group A

Variables	Preoperatively			Postoperatively			t test	P value
	N	Mean	±SE	N	Mean	±SE		
PD	10	1.20	0.1333	10	0.50	0.1667	4.58	0.0013*
CAL	10	4.00	0.2582	10	1.00	0.2582	14.23	0.0000*
RH	10	2.80	0.2000	10	0.50	0.1667	15.06	0.0000*
RW	10	3.00	0.2108	10	0.30	0.1528	17.68	0.0000*
WKT	10	4.30	0.3350	10	6.60	0.3055	15.06	0.0000*

N=number; SE=standard deviation error; mm=millimetre; PD=probing depth; CAL=clinical attachment level; RH=recession height; RW=recession width; WKT=width of keratinized tissue.

*P value less than 0.05 is considered statistically significant.

Comparison of mean baseline and three-month postoperative values of clinical periodontal parameters in Group A

The mean probing depth was 1.20 ± 0.13 mm at baseline and 0.30 ± 0.15 mm at three months after surgery, with a p value of 0.0007. The mean CAL was 4.00 ± 0.21 mm at baseline and 0.60 ± 0.16 mm at three months after surgery, with a p value of 0.0000. The mean difference in RH was 2.50 ± 0.10 mm, with a p value of 0.0000. The mean difference in recession width was 2.40 ± 0.12 mm at three months postoperatively, with a p value of 0.0000. The mean gain in the WKT was 2.50 ± 0.27 mm, with a p value of 0.0000. Comparisons of baseline and three-month postoperative values of Group A showed that the differences were statistically significant. The data are listed in Table 2.

Comparison of mean baseline and three-month postoperative values of clinical periodontal parameters in Group B

The mean reduction in the probing depth was 0.70 ± 0.01 mm, with a p value of 0.0013. The mean gain in the CAL was 3.00 ± 0.00 mm, with a p value of 0.0000. The mean difference in the RH was 2.30 ± 0.03 mm, with a p value of 0.0000. Reduction in recession width at

TABLE 3. Comparison of mean preoperative and postoperative values of various clinical parameters in Group B

Variables	Preoperatively			Postoperatively			t test	P value
	N	Mean	±SE	N	Mean	±SE		
PD	10	1.20	0.1333	10	0.50	0.1667	4.58	0.0013*
CAL	10	4.00	0.2582	10	1.00	0.2582	14.23	0.0000*
RH	10	2.80	0.2000	10	0.50	0.1667	15.06	0.0000*
RW	10	3.00	0.2108	10	0.30	0.1528	17.68	0.0000*
WKT	10	4.30	0.3350	10	6.60	0.3055	15.06	0.0000*

N=number; SE=standard deviation error; mm=millimetre; PD=probing depth; CAL=clinical attachment level; RH=recession height; RW=recession width; WKT=width of keratinized tissue.

*P value less than 0.05 is considered statistically significant.

TABLE 4. Comparison of mean postoperative values of clinical parameters in Groups A and B

Variables	Group A			Group B			t test	P value
	N	Mean	±SE	N	Mean	±SE		
PD	10	0.30	0.1528	10	0.50	0.1667	0.88	0.3880
CAL	10	0.60	0.1633	10	1.00	0.2582	1.31	0.2069
RH	10	0.30	0.1528	10	0.50	0.1667	0.88	0.3880
RW	10	0.10	0.1000	10	0.30	0.1528	1.10	0.2878
WKT	10	6.90	0.2769	10	6.60	0.3055	0.73	0.4762

N=number; SE=standard deviation error; mm=millimetre; PD=probing depth; CAL=clinical attachment level; RH=recession height; RW=recession width; WKT=width of keratinized tissue.

*P value less than 0.05 is considered statistically significant.

three months was 2.70 ± 0.06 mm, with a p value of 0.0000. The mean gain in the WKT was 2.30 ± 0.02 mm, with a p value of 0.0000. Comparisons of baseline and three-month postoperative values of Group B showed that the differences were highly statistically significant. The data are listed in Table 3.

Comparison of mean three-month postoperative values of clinical periodontal parameters in Groups A and B

The mean three-month postoperative probing pocket depth was 0.30 ± 0.15 mm for Group A and 0.50 ± 0.17 mm for Group B, with a p value of 0.3880. The mean three-month postoperative CAL was 0.60 ± 0.16 mm for Group A and 1.00 ± 0.26 mm for Group B, with a p value of 0.2069. The mean three-month postoperative recession height was 0.30 ± 0.15 mm for Group A and 0.50 ± 0.17 mm for Group B, with a p value of 0.03880. The mean three-month postoperative recession width was 0.10 ± 0.10 mm for Group A and 0.30 ± 0.15 mm for Group B, with a p value of 0.2878. The mean three-month postoperative value of WKT was 6.90 ± 0.28 mm

for Group A and 6.60 ± 0.31 mm for Group B, with a p value of 0.4762. Comparisons of the mean three-month postoperative values showed statistically non-significant differences between Groups A and B. The data are listed in Table 4.

Mean root coverage (MRC)

The percentage of root coverage (PRC) was calculated postoperatively by using the following formula:

Root coverage = preoperative recession height – postoperative recession height/preoperative recession height $\times 100$.

% coverage = (Pre-RH)-(post RH) $\times 100$ /(Pre-RH)

The mean value of root coverage was 89% in Group A and 82% in Group B at three months after surgery. □

DISCUSSION

Periodontal surgery aims to achieve the functional goals and esthetic concern of patients. Gingival recessions account to a majority of esthetic concerns associated with periodontal tissue deformities (6). Various factors, including dehiscence of bone, insufficiency of keratinized tissue, mal positioning of the tooth, high frenal attachment and inadequate vestibular depth, also lead to recession of the gingival tissues. Regressive alterations of the tooth structure such as abrasion, abfraction and erosion, plaque and calculus accumulation trigger inflammation due to improper or traumatic brushing techniques, which are the major factors that induce gingival recessions.

Other iatrogenic factors such as ill-fitting dentures, traumatic operative procedures and occlusal trauma may also cause gingival tissue recession. Diverse factors that may lead to gingival recession include age, gender, piercing and chewing stick trauma (Nguyen-Hieu, *et al*, 2012) (7). The appropriate treatment method of gingival recession management fully depends upon the etiology and tissue loss. The first step in treating gingival recession is the elimination of the etiology and aggravating factors that induce gingival recession. Once the cause is eliminated, the amount of tissue loss and exposed root surface determines the surgical technique. Various treatment modalities for gingival recession comprise advanced flap (coronally advanced flap), pedicle grafts (lateral pedicle and its modifica-

tions), free gingival and connective tissue graft. A systematic review published by Cairo *et al* in 2008 concludes that coronally advanced flap is a safe and more predictable method for the management of gingival recessions (8).

In 2015, Rose *et al* were the first to describe the presence of growth factors in platelets (9). Growth factors are large polypeptide molecules obtained autogenously or prepared by recombinant technologies. Platelets are one of the best sources of autogenous growth factors. In 2001, Clark *et al* proved that growth factors released from local applications enabled better tissue regeneration by having a major role in cell proliferation, survival, migration and angiogenesis (10).

The molecular and cellular events which take place during a traumatic injury are mostly the same in every tissue of the body that involves the evoking of inflammation phases, migration of cells, angiogenesis, formation of granulation tissue, matrix formation and cellular proliferation (11). These events are stimulated by growth factors, cytokines and proteins from the serum and degranulating platelets.

Membranes of the human placentas have been used in the successful management of burns and skin ulcers in the field of medicine. The most recent development in dentistry is the use of placental allografts, with the first commercial product being available in 2008. Human placental membranes enhance wound healing and may stimulate regeneration of the lost periodontal tissues because of their unique biologic properties. One such placental membrane is the chorion membrane, which has various advantages in treating gingival recession due to its structure and composition. The structure of the chorion membrane comprises a reticular layer, basement membrane and trophoblasts (12). The composition of the chorion membrane consists of an extracellular matrix comprising collagen types I, III, IV, V and VI, and cell-adhesion bioactive factors (fibronectin and laminin) (13).

The collagen of the chorion membrane is well tolerated and bioabsorbable. Also, it has hemostatic properties and induces migration of adjacent connective tissues. Fibronectin plays a major role in tissue repair, stabilization of blood clot, migration and cell adhesion (14). Laminin has more affinity for binding epithelial cells. Chorion membrane allows for rapid epithelial cell growth. Also, there are abundant growth factors in the

matrix of the chorion, including keratinocyte growth factor, basic fibroblast growth factor and transforming growth factor- β , which have a major role in promoting periodontal regeneration (15) and provide a better healing environment for the periodontal tissues.

Recent research is focused on the application of autogenous growth factors such as platelet rich fibrin and concentrated growth factor in periodontal regeneration techniques. However, the phases of tissue repair are mediated by the growth factors and cytokines produced locally at the site of inflammation, which modulates the cellular function through direct physical interaction with the extracellular domain of transmembrane receptors. The platelet derived growth factor (PDGF) acts as a power mitogen in the connective tissue cells, and stimulation of osteoprogenitor cells is mediated by the transformation growth factor- β (TGF- β). The late-stage differentiation of osteoblasts is promoted by the insulin like growth factor and endothelial migration, and proliferation is initiated by the vascular endothelial growth factor (VEGF) thus promoting angiogenesis.

The present study was done to compare and evaluate the effectiveness of human chorion membrane and concentrated growth factor membrane in the treatment of Miller's Class I and II bilateral gingival recessions in maxillary premolars. Results show that both chorion membrane and concentrated growth factor have the ability to reduce probing depth, increase clinical attachment level and have given sufficient root coverage. □

CONCLUSION

Root coverage procedures done with coronally advanced flap with human chorion membrane and concentrated growth factor membrane as an adjunct can have a successful outcome in root coverage, with enhanced healing and regeneration of the periodontal tissues along with increase in gingival biotype. In the future, more studies are required to prove the effectiveness of chorion and concentrated growth factor as membrane in regeneration of periodontal tissues. □

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