



## **Modified Coronally Advanced Tunnel Technique in Conjunction with Either Platelet Rich Fibrin or Chorion Membrane in the Treatment of Gingival Recession: A Split Mouth Study.**

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### **Abstract:**

**Background:** *Varying surgical techniques in conjunction with different regenerative materials have been introduced to obtain predictable root coverage. The objective of this study was to clinically evaluate and compare the efficacy of platelet-rich fibrin (PRF) with that of chorion membrane (CM) in the treatment of gingival recession. Both the membranes were used in conjunction with the modified coronally advanced tunnel technique (MCAT).*

**Methodology:** 14 patients with a total of 28 gingival recession defects were selected and randomly assigned either to experimental site-A (MCAT with PRF) or experimental site-B (MCAT with CM). Clinical parameters such as plaque index (PI), gingival index (GI), recession depth (RD), probing depth (PD), clinical attachment level (CAL), width of keratinized tissue (WKT) and gingival thickness (GT) were recorded at baseline and 9 months postoperatively. ANOVA, Tukey POST HOC, and unpaired t-test were used to assess statistical significance ( $p < 0.01$ ).

**Results:** Mean RD significantly decreased from baseline to 9 months in both groups. No statistically significant differences between the two groups were found for RD, CAL, PD and WKT gain. Though both the procedures resulted in an enhancement of the GT, MCAT with CM resulted in a significant increase in the gingival thickness at the 9 months postoperative evaluation when compared to MCAT with PRF. **CONCLUSION:** Within the limits of the study modified coronally advanced tunnel technique in conjunction with either platelet-rich fibrin or chorion membrane resulted in a significant amount of root coverage and also enhancement of the gingival biotype which may lead to the long-term stability of the clinical outcomes.

**Keywords:** root coverage, MCAT, chorion membrane, PRF, gingival biotype

## Introduction

Gingival recession (GR) is defined as the exposure of the root surface due to the displacement of the gingival margin apical to the cemento-enamel junction (CEJ). It may be localized or generalized and can be associated with one or more tooth surfaces. The exposed root surfaces are frequently associated with esthetic complaints, root hypersensitivity and difficulties in achieving optimal plaque control.<sup>1</sup>

The etiology of the condition is multifactorial and includes over contoured tooth shape, thin biotype, malposition of teeth, ectopic insertion of the frenum, muscle attachments, improper oral hygiene methods, localized or generalized periodontal disease, or iatrogenic dental treatments. As one of the significant predeterminants, a thin gingival biotype is considered to be the most relevant anatomical factor of gingival recession.<sup>1</sup>

An array of therapeutic options are available for management of gingival recession which not only aims at obtaining complete root coverage and esthetics but also improve the gingival thickness, an important qualitative parameter for long term stability.<sup>2</sup> Esthetic results for root coverage and gains in clinical the attachment has been reported for coronally positioned flaps in combination with connective tissue grafts, making it the standard technique for root coverage. However, the coronally positioned flap requires

vertical releasing incisions on the buccal side, which may jeopardize the esthetic result. To avoid these incisions, the tunnel technique has been developed and further modified.<sup>3</sup>

The modified coronally advanced tunnel the technique (MCAT) was developed by Azzi and co-workers in 2002 and it offers the advantage of avoiding vertical incisions and incising the papilla which improves the vascularisation of the surgical area and allows a tension-free coronal displacement of the flap. This technique can be used in the treatment of both isolated, as well as multiple adjacent gingival recession, defects.<sup>4</sup>

The predictability of surgical outcomes can be increased by using regenerative materials in conjunction with surgical procedures.

Platelet-rich fibrin (PRF), a second-generation platelet concentrated suspension of growth factors was first developed by Choukron et al (2001). A PRF membrane consists of a fibrin 3D matrix polymerized in a specific structure, with the incorporation of platelets, leukocytes, growth factors and circulating stem cells.<sup>5</sup> It can be obtained from autologous blood with simplified processing without the need for biochemical blood handling. It has become a focus of current studies because of its potential to accelerate healing<sup>6</sup> and has been postulated as a promoter of tissue regeneration.<sup>7</sup>

One of the biomaterials used for scaffolds is the fetal membrane which comprises of the amniotic and chorion tissues. The chorion forms the outer limits of the sac that encloses the fetus and comprises an extracellular matrix composed of different types of collagen, cell adhesion bioactive factors and growth factors. These properties suggest that the chorion membrane, an immunoprivileged tissue may have considerable potential for regeneration.<sup>8</sup>

Hence, the purpose of the present clinical the study was to compare and evaluate clinically the efficacy of modified coronally advanced tunnel technique in conjunction with either platelet-rich fibrin or chorion membrane in the treatment of Miller's class I or class II gingival recession defects.

## **Material and Method**

A total of 14 systematically healthy patients within the age range of 20-60 years were enrolled in the study after obtaining written consent. The subjects were included in the study based on the following criteria: a) were non-smokers b) Teeth involved in the study showed the presence of Miller's class I or class II gingival recession defects measuring  $\geq 2$ mm on the anterior teeth or premolars, on contralateral sides of the same arch or one in each quadrant, c) no history of surgical treatment in the delineated area for at least 2 years before the study d) teeth involved were vital, free of faulty restorations and had no signs of trauma from occlusion. They were excluded if they showed the presence of any uncontrolled local or systemic disease that might contraindicate periodontal surgery.

The chorion membrane (CM) in the size, 3\*3 cm was obtained from the Tata memorial hospital tissue bank, Mumbai, India.

Before surgery, all the patients received professional oral prophylaxis, oral hygiene instructions, and occlusal adjustments as per their requirements.

**Clinical parameters:**

A customized acrylic stent was made with guiding grooves on each experimental tooth angled towards the deepest part (ie, the midfacial part) of the recession. One trained examiner performed all the clinical measurements on the mid buccal aspect of the gingival recession defects using a UNC-15 periodontal probe and the acrylic stent. Any measurement falling in the 1mm interval was considered as 0.5mm. Baseline measurements were taken before the procedure.

The clinical parameters assessed include plaque index (PI) using Loe and Silness 1964 and gingival index (GI) using Silness and Loe 1963. Probing pocket depth (PD) was measured from the gingival margin to the base of the gingival sulcus, Vertical depth of the recession (RD) was measured from the cemento-enamel junction (CEJ) to the gingival margin and clinical attachment level (CAL) was measured from the CEJ to the base of the gingival sulcus. Keratinized tissue width (KTW) was measured from the mucogingival junction to the gingival margin Percentage of root coverage was calculated as

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

The gingival thickness (GT) was measured after anesthetizing the surgical site. The measurements were assessed mid buccally on the attached gingiva, halfway between the mucogingival junction and free gingival groove using an endodontic spreader and rubber stopper. Those markings were then read using a digital vernier caliper. The gingival biotype was considered thin if the measurement was  $\leq 1.0$  mm and thick if it measured  $\geq 1.0$  mm as described earlier by Kan et al. All the measurements were made at baseline and 9 months follow-up.

**Surgical Procedure:**

After the baseline measurements were recorded, the contralateral sites were randomly assigned to one of the surgical techniques (MCAT with PRF or chorion membrane) by the coin flip method. After extraoral preparation with 5% povidone-iodine solution, the patient was asked to rinse with 10ml of 0.2% chlorhexidine digluconate solution for one minute. The surgical site was then anesthetized by local infiltration (2% lidocaine with adrenaline 1:80,000).

**Site treated with modified coronally advanced tunnel and chorion membrane**

Following local anesthesia, root planing of the exposed root surface was performed with Gracey curettes. Intrasulcular incisions not involving the interdental papillae around the involved tooth were performed. A mucoperiosteal flap was separated beyond the level of the mucogingival junction leaving interdental papillae intact with the use of periostomes. Subsequently, the mucoperiosteal tunnel was extended by full-thickness preparation laterally from the recession. It is important to extend the tunnel elevation sufficiently beyond the mucogingival margin as well as through the gingival sulci of the teeth being augmented to allow for low-tension coronal repositioning of the gingiva. Finally, to achieve complete mobilization of the flap, the interdental papillae were gently undermined.<sup>1</sup> Subsequently, the chorion membrane was trimmed and carefully advanced into the tunnel. The membrane and mucogingival complex was then advanced coronally and stabilized in the new position with a coronally anchored suturing technique.<sup>9</sup> (Figure III)

**Platelet-rich fibrin (PRF) Preparation:**

The PRF was prepared following the protocol developed by Choukron et al. Just before surgery, intravenous blood (by venipuncture the antecubital vein) was collected in a 10ml sterile tube without anticoagulant and immediately centrifuged in a centrifugation machine at 3,000 revolutions per minute for 10 minutes. Blood centrifugation immediately after collection allowed for the composition of a structured fibrin clot in the middle of the tube just between the red corpuscles at the bottom and acellular plasma (platelet-poor plasma) at the top. The PRF was easily separated from the red corpuscles base (presenting a small red blood cell [RBC]) layer using sterile tweezers and scissors just after the removal of platelet-poor plasma and then transferred onto a sterile compress. A stable fibrin membrane was obtained by squeezing serum out of PRF clot.<sup>6</sup> (FIGURE I a and b)

**Site treated with modified coronally advanced tunnel and Platelet-rich fibrin**

**(PRF) membrane**

A similar surgical technique as described earlier was performed on the other site which was treated with modified coronally advanced tunnel and PRF membrane. Once coronal advancement of the gingival margin was established, the freshly prepared PRF membrane was trimmed and inserted into the tunnel with the periostome. The stabilization of the membrane was achieved by the gentle pressure for 3 minutes. Later, coronally anchored bonded sutures were placed. (FIGURE II)

**Statistical Analysis:**

Data was analyzed using SPSS version 23. A p-value of <0.05 was considered statistically significant. ANOVA and Tukey post-hoc the test was done to compare parameters from baseline through nine

months follow-up. The unpaired t-test was used to compare differences in the follow-up between sites A and B.



**FIGURE I(a):** i.v Blood Collection



**FIGURE I(b):** PRF preparation

**Figure II: Experimental Site A**



**FIGURE II(a):** Pre-operative view of the site



**FIGURE II(b):** Tunnel preparation



**FIGURE II(c):**PRF Membrane Secured on the Recipient Site



**FIGURE II(d):** Coronally repositioned tunnel using coronally anchored sutures



**FIGURE II(e):**Nine-month post-operative view showing stable root coverage

**Fig III: Experimental Site B**



**FIGURE III(a):** Pre-operative view of the site



**FIGURE III(b):** Tunnel preparation



**FIGURE III(c):** Sterile chorion membrane trimmed and contoured



**FIGURE III(d):** Chorion membrane secured on the recipient site



**FIGURE III(e):** Coronally repositioned tunnel using coronally anchored sutures



**FIGURE III(f):** Nine-month post-operative view showing stable root coverage

## Result

Fourteen patients of mean age  $32.64 \pm 7.10$  years fulfilling the inclusion criteria were selected for the study. Twenty-eight sites in fourteen patients were randomly divided into experimental site A (MCAT using Platelet Rich Fibrin) and experimental site B (MCAT using chorion membrane). Uneventful healing with no post-operative complications was experienced in all patients.

The mean PI at experimental site A was recorded as  $0.80 \pm 0.17$ . It reduced to  $0.11 \pm 0.13$  at 9 months. In experimental site B, it reduced from  $0.88 \pm 0.27$  at baseline to  $0.14 \pm 0.13$  at 9 months. In both the sites the reduction in the PI scores from baseline to 9 months was statistically significant ( $p < 0.5$ ). The mean gingival index recorded at baseline for experimental sites A and B was  $0.00 \pm 0.00$ . It increased to  $0.05 \pm 0.11$  in site A and  $0.02 \pm 0.07$  in site B at 9 months follow up. The difference in the mean GI scores obtained at baseline and 9 months in both the sites was not statistically significant.

The mean reduction in the PD from baseline to 9 months was  $0.57 \pm 0.11$  mm in experimental site A, which was statistically significant ( $p < 0.01$ ). Similarly, in experimental site B a significant PD reduction of  $0.29 \pm 0.09$  mm was obtained from baseline to 9 months ( $p < 0.01$ ). The mean gain in the CAL from baseline to 9 months was  $2.21 \pm 0.25$  mm in site A and  $2.29 \pm 0.06$  mm in site B, which was statistically significant ( $p < 0.01$ ).

On comparing the two sites at baseline and 9 months there was no statistical significance for PI, GI, PD and CAL ( $p > 0.05$ ).

The mean RD at baseline in site A was  $2.14 \pm 0.36$  mm. It reduced to  $0.36 \pm 0.50$  mm at 9 months. Similarly, in site B the mean RD at baseline was  $2.50 \pm 0.52$  mm which reduced to  $0.43 \pm 0.65$  mm at 9 months. A significant reduction in the mean RD from baseline 9 months was observed in both the groups ( $p < 0.05$ ). The mean percentage of root coverage obtained at 9 months from baseline was  $82.14 \pm 0.61\%$  in experimental site A and  $84.51 \pm 0.53\%$  in experimental site B.

Table I and II illustrates that the mean KTW in both the groups was  $1.93 \pm 0.27$  mm at baseline which increased to  $3.21 \pm 0.43$  mm in group A and  $3.29 \pm 0.47$  mm in group B at 9 months, which was statistically highly significant ( $p < 0.01$ ). However, the intergroup comparison showed a non-significant difference from baseline to 9 months for RD, mean percentage of RC and KTW ( $p > 0.05$ ).

In Table I and II, both group A and group B show a statistically significant increase in GT from  $0.94 \pm 0.27$  mm to  $1.71 \pm 0.43$  mm and  $0.94 \pm 0.22$  mm to  $2.02 \pm 0.24$  mm at 9 months follow up. However, on intergroup comparison the increase in GT was significantly greater in group B than group A at 9 months ( $p < 0.5$ ).

Clinical parameter	Site A		Site B		p- value	Inference
	Mean	SD	Mean	SD		
GI	0	0	0	0	0	-
PI	0.80	0.17	0.88	0.27	0.42	NS
RD (in mm)	2.14	0.36	2.50	0.52	0.62	NS
PD (in mm)	1.86	0.36	1.50	0.52	0.38	NS
CAL (in mm)	4.00	0.55	4.00	0.55	1	NS
WKT (in mm)	1.93	0.27	1.93	0.27	1	NS
GT (in mm)	0.94	0.27	0.94	0.27	0.94	NS

NS: non-significant

**Table I:** Comparison between experimental site A and B at baseline

Clinical parameter	Site A		Site B		p- value	Inference
	Mean	SD	Mean	SD		
GI	0.05	0.11	0.02	0.07	0.30	NS
PI	0.11	0.13	0.14	0.13	0.47	NS
RD (in mm)	0.36	0.50	0.43	0.65	0.75	NS
PD (in mm)	1.29	0.47	1.21	0.43	0.68	NS
CAL (in mm)	1.79	0.80	1.71	0.61	0.79	NS
WKT (in mm)	3.21	0.43	3.29	0.47	0.67	NS
GT (in mm)	1.72	0.43	2.04	0.25	<0.05	S

S: significant

**Table II:** Comparison between experimental site A and B at 9 months follow-up

## Discussion

The results of this study demonstrated that modified coronally advanced tunnel technique in conjunction with either platelet rich fibrin or chorion was effective in the treatment of gingival recession defects ( $\geq 2\text{mm}$ ). Both sites showed significant root coverage (82.14% and 84.51% respectively), gain in the width of keratinized gingiva and clinical attachment gain at 9 months post operatively. The difference between the two procedures in terms of reduction in recession depth and probing depth are not statistically significant. However, sites treated with modified coronally advanced tunnel in conjunction with chorion showed greater increase in the gingival thickness/biotype.

Critical to the success of the MCAT technique is a careful subperiosteal dissection extended beyond the mucogingival junction, under each papilla and lateral to the recession site. The collagen bundles which prevent the flap from being coronally advanced are released with currettes to obtain an effect similar to

that of a horizontal releasing incision. This reduces the tension of the gingival margin during coronal advancement, while at the same time maintaining the anatomical integrity of the interdental papillae by avoiding papillary reflection. The absence of vertical incisions may in turn improve the revascularization of the area.<sup>10</sup>

Coronally anchored bonded sutures are retained for three weeks to allow for immobilization of the gingival margin during the initial phases of healing. The gingival margin, with the membrane, is advanced to the most coronal level of the adjacent interproximal papillae rather than to the cemento-enamel junction. Vicryl sutures are then secured to the facial aspect of each tooth, effectively preventing apical relapse of the gingival margin during the initial stages of healing but compensating for some degree of apical migration during the healing period. Apical migration of the gingival margin over relatively long periods of follow-up appears either minimal or nonexistent with this tunnel procedure. The rigid fixation of gingival margins used with the present coronally anchored suturing technique minimizes micromotion at the regenerative site. Reduction of micromotion has proven to be a major advantage of the present technique over conventional methods, where the gingival margin may be subjected to displacement during facial movement.<sup>9</sup>

Although considered the current gold standard, the connective tissue graft presents a number of limitations, including the need for harvesting at a distant donor site, limited tissue availability, and increased potential for post harvesting morbidity. Additionally, larger grafts impair the vascular exchange between the covering flap and the underlying recipient bed, thus increasing the risk of flap dehiscence and causing unesthetic graft exposure as stated by Zucchelli et al in his classical study in 2009.<sup>11,12</sup>

To overcome these inconveniences, attempts have been made to develop new materials aiming to replace connective tissue graft to improve patient acceptance and minimize morbidity.

The scientific rationale behind the use of platelet preparations lies in the fact that the platelet  $\alpha$ -granules are a reservoir of many growth factors that are known to play a crucial role in hard and soft tissue repair mechanism. These include platelet-derived growth factors (PDGFs), transforming growth factor beta (TGF- $\beta$ ), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin like growth factor-1 (IGF-1). PRF organizes as a dense fibrin scaffold with a high number of leukocytes concentrated in one part of the clot, with a specific slow release of growth factors for more than 7 days after its placement.

Platelets also play a role in the host defense mechanism at the wound site by delivering signaling peptides that attract macrophage cells. In addition, platelet concentrates may contain small amounts of leukocytes that synthesize interleukins involved in the non-specific immune reaction. The antimicrobial activity of platelet concentrates against several bacterial species involved in oral infections has also been reported.<sup>13</sup>

The potential advantage of utilizing biomaterials in root coverage procedures is the possibility of achieving periodontal regeneration rather than connective tissue repair to the exposed root surface. One such biomaterial is the foetal membrane. Chorion which is the outer layer of the foetal sac has numerous advantages owing to its structure and composition. This allograft comprises of an extracellular matrix rich in collagen type I, IV, V and VI and cell-adhesion bioactive factors such as fibronectin and laminin. Chorion membrane when compared with the amniotic membrane showed greater rigidity and better handling properties.<sup>8</sup>

Collagen is well-tolerated and bio-absorbable, has hemostatic properties, and encourages migration of cells from the adjacent autogenous connective tissue. Laminins exhibit a variety of biologic activities, including promotion of cell attachment, growth, and differentiation of a number of cell types. Fibronectin is involved in many cellular processes including tissue repair, blood clotting, cell migration and adhesion. Additionally, the matrix contains growth factors such as keratinocyte growth factor, basic-fibroblast growth factor, transforming growth factor-beta which promote periodontal regeneration and provide a natural environment for accelerated healing.<sup>14</sup>

At baseline, site A and B demonstrated non-significant difference in plaque levels and gingival findings. There was a significant decrease in both the scores from baseline to 9 months. This indicates that the patients had been motivated well enough to maintain their oral hygiene properly.

A statistically significant ( $p < 0.01$ ) reduction in mean recession depth of  $1.78 \pm 0.14$  mm was obtained at 9 months in sites treated with MCAT + PRF.

Unlike other autologous platelet concentrates PRF proves to be advantageous as there is increased incorporation of the circulating cytokines in the fibrin mesh with an increased life span thereby creating a long term effect. In PRP and other fibrin adhesives the presence of artificial additives results in sudden polymerisation of fibrin causing loss of synergy between the cytokines leading to faster physiologic elimination of these cytokines.

The three dimensional organization of a natural fibrin network in PRF is more elastic and flexible favouring cellular migration. These features make it a better healing biomaterial.<sup>5</sup>

A statistically significant ( $p < 0.01$ ) reduction in mean recession depth of  $2.07 \pm 0.13$  mm, a mean root coverage of  $84.51 \pm 0.53\%$  and 100% root coverage was seen in 9 out of 14 sites treated with modified coronally advanced tunnel technique with chorion membrane at the end of 9 months.

Chorion membrane (CM) is available in different dimensions, sufficient enough to adapt to the site as a single strip, with trimming at the borders. The barrier is placed dry and quickly hydrates with blood, becomes very pliable, and closely adapts to the contours of the underlying surface. It's thin, self adherent nature does not compromise blood flow. Chorion membrane will not easily displace from underneath

the overlying flap and does not require being fixed into place using sutures or tacks. This property of self-adherence can be contributed to the presence of various cell adhesion molecules such as laminins.<sup>15</sup>

Immunohistochemical staining analysis of Chorion membranes showed intense concentrations of laminin and laminin-5 throughout the barrier (Bryant-Greenwood 1998). Laminin-5 is of particular importance due to its high affinity for binding gingival epithelial cells for better adaptation to the root surface (Mullen et al. 1999) Collectively, CM's unique biologic and physical attributes reduce the complexity of trimming, suturing and placement of barriers, minimizing the chances of post-operative complications (Holtzclaw and Toscano 2012).<sup>15</sup>

Both the sites showed statistically significant gain. Comparison of the two sites showed that there was no statistical significance between them. A mean increase of  $1.28 \pm 0.16$  mm from baseline recording, was found in sites treated with modified coronally advanced tunnel technique in conjunction with platelet rich fibrin which was statistically highly significant ( $p < 0.01$ ). Notably, gain in the keratinized tissue in the sites treated with PRF may be explained as a result of a tissue manifestation of the proliferation of fibroblasts as a result of the influence of the growth factors from platelets entrapped in a fibrin mesh. However, further scientific evidence is required.<sup>5</sup>

A mean gain of  $1.36 \pm 0.20$  mm in the width of keratinized gingiva from baseline recordings was found in sites treated with modified coronally advanced tunnel technique with chorion membrane, which was statistically highly significant ( $p < 0.01$ ). It is known that, once stability of the soft tissue margin has been obtained at the level of the CEJ, the keratinized tissue is able to increase with time. The significant gain in width of keratinized tissue may be attributed to the presence of mitogenic factors and anti-inflammatory proteins.<sup>31</sup> The tendency of the mucogingival line to regain its "genetically" determined position following coronal dislocation with the flap procedure may also contribute to the of the increased apicocoronal gingival height.<sup>16</sup>

A statistically significant mean reduction in probing depth of  $0.57 \pm 0.11$  mm and  $0.48 \pm 0.22$  mm, was found at sites treated with modified coronally advance tunnel with PRF and chorion membrane respectively. The mean gain in clinical attachment level was 2.21 and 2.29 mm respectively. Since no histological evaluations were available, the type of healing obtained following the procedures can only be speculated on. This gain probably represents a combination of the formation of new connective tissue attachment and epithelium attachment.

This may be due to the presence of tissue inhibitor of metalloproteinases (TIMPs) in chorion membrane which suppresses matrix metalloproteinases (MMPs) and transforming growth factor beta (TGF- $\beta$ ) which stimulates the production of TIMPs from the surrounding tissue (Hao et al. 2000; Riau et al. 2010). Collectively, these proteins suppress inflammation and collagenous degradation; also, the presence of intense concentrations of laminin and laminin-5 throughout the barrier is of particular importance due

to its high affinity for binding gingival epithelial cells, which may contribute for better adaptation to the root surface (Pakkala et al. 2002).<sup>15</sup>

Gingival thickness can also be measured by other non-invasive procedures such as ultrasonic devices and cone-beam computed tomography. Although the former method is more comfortable for the patient, the studies by Eger T et al 1996 and Muller Hp et al 1999 reported difficulty in obtaining reliable results on a consistent basis. The latter method using CBCT reveals a high quality image of hard and soft tissue and allows measurements of the dimensions and relationships of these structures. However, this method is unable to distinguish between normal and inflamed gingiva, which have similar images acquired by CBCT.<sup>17</sup>

The present study has reported an increase in gingival thickness in sites treated with MCAT and platelet rich fibrin from  $0.94 \pm 0.27$ mm to  $1.71 \pm 0.43$  mm at 3 months and this remained unchanged at 6 month and 9 month follow up. The gingiva has been enhanced to a thick biotype.

Studies by Corso et al (2009) and Aroca S et al (2009) have found that treatment of gingival recession with PRF resulted in significant improvement during the early periodontal healing phase with a thick and stable final remodelled gingiva. This may be the result of gingival and periodontal fibroblast proliferation related to the impact of concentrated growth factors from PRF.<sup>5</sup>

At sites treated with modified coronally advanced tunnel and chorion membrane the mean gingival thickness at baseline was  $0.94 \pm 0.22$  mm which improved to  $2.02 \pm 0.24$ mm at 3 months and was maintained at 6 month and 9 month follow up. This improvement in gingival thickness obtained in these sites was significantly higher than those obtained in the sites treated with PRF.

The increase in gingival thickness using chorion membrane may be due to the presence of a large number of pro-angiogenic growth factors, including angiogenein, angiopoietin-2, epidermal growth factor (EGF), fibroblast growth factor (bFGF), hepatocyte growth factor (HB-ECF, HGF), platelet derived growth factor (PDGF-BB), placental growth factor (PGF) and vascular derived growth factor (VEGF). These growth factors promote endothelial recruitment and better vascularisation, ensuring a better blood supply to the flap connective tissue (Koob et al. 2014).<sup>18</sup>

#### **Limitations of the study:**

1. Histological assessment of the type of attachment was not done due to ethical considerations.
2. Most of the gingival recession defects treated fall under shallow recession group with a recession depth around 2-3mm. Hence the treatment results are not conclusive.

In conclusion, within the limits of the present study, it has been demonstrated that modified coronally advanced tunnel technique in conjunction with either platelet-rich fibrin or chorion membrane was

effective in the treatment of gingival recession not only by providing predictable root coverage but also augmenting the the gingival thickness that resulted in long term stability.

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