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Original Research Article

Comparative evaluation of the efficacy of coronally repositioned flap with PRF and with placental membrane allograft (Amniotic Membrane) in the treatment of gingival recession

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ABSTRACT

Introduction: Gingival recession presents a significant aesthetic problem in dental patients. A variety of surgical techniques have been used to cover these defects.
Materials and Methods: The present study was carried out on 30 selected sites having either Miller's Class I or II marginal tissue recession. This is a clinical study designed as a split mouth randomized controlled trial. Each site constitutes one sample of the study. 15 sites were randomly assigned for the test group (Coronally Advanced Flap with Amnion membrane) and another 15 sites were assigned for the control group (CAF with PRF). All subjects received clinical periodontal examination by a single examiner.
Result: There was a statistically non significant difference seen for the values between the inter and intra groups ($p>0.05$) for RD at all time intervals.
Conclusion: Both the treatment modalities for gingival recession coverage demonstrated a significant improvement in the recession coverage and width of keratinized gingiva and can be used in treatment of gingival recession defects.

1. Introduction

The objective of any therapeutic intervention of root coverage is to restore the marginal tissue to the cement enamel junction. Gingival recession has been defined as the term used to characterize the apical shift of the marginal gingiva from its normal position on the crown of the tooth to levels on the root surface beyond the Cemento-Enamel Junction (CEJ).[1] In most cases the apical shift of the marginal tissue does not extend beyond the Mucogingival Junction, but in cases where the tissue has receded into the alveolar mucosa, marginal tissue recession may be a better and more precise term.[2]

The etiology is multi factorial and may include accumulation of dental plaque biofilm with the resulting inflammatory periodontal diseases and mechanical trauma due to faulty brushing technique.[3] Several other risk factors play a role in the occurrence of recession, including aging, alveolar bone dehiscence, high frenum attachment, and smoking. Other factors include calculus, restorative, iatrogenic factors, tooth malpositions, improper periodontal

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treatment procedures, and uncontrolled orthodontics movements.[4]

Till date subepithelial connective tissue graft with or without Coronally Advanced Flap(CAF) has been considered to be gold standard for treatment of gingival recession. However, this is a technique sensitive procedure and often associated with increased postoperative patient complication.[5] The time involved in the surgical procedure is also prolonged. Thus, periodontists around the world have been evaluating coronally advanced flap along with materials like PRF, EMD, GTR membrane and other materials in place of sub epithelial connective tissue graft.

Platelet-rich fibrin (PRF) described by Choukroun et al. is a second-generation platelet concentrate which contains platelets and growth factors in the form of fibrin membranes prepared from the patient's own blood free of any anticoagulant or other artificial biochemical modifications. The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest and shows a complex architecture as a healing matrix with unique mechanical properties which makes it distinct from other platelet concentrates. PRF enhances wound healing, regeneration and several studies show rapid and accelerated wound healing with the PRF than without it. PRF is superior to other platelet concentrates like PRP due to its ease and inexpensive method of preparation and also it does not need any addition of exogenous compounds like bovine, thrombin and calcium chloride. It is advantageous than autogenous graft because an autogenous graft requires a second surgical site and procedure. Thus, PRF has emerged as one of the most promising regenerative materials in the field of periodontics. This article explains the novel platelet concentrate PRF, its preparation, clinical applications, benefits and drawbacks over other biomaterials.[6]

Recently, the efficacy of amniotic membrane was evaluated along with CAF for root coverage. Human amniotic membrane is the innermost layer of the placenta which lines the amniotic cavity. It is composed of a single layer of epithelial cells, a basement membrane, and an avascular connective tissue matrix. The basement membrane contains collagen Types III, IV, V, and cell-adhesion bioactive factors including glycoproteins, fibronectin, and laminins (laminin-5) plays a role in the cell adhesion of gingival cells. It also contains stem cells and growth factors such as epidermal growth factor, transforming growth factor beta, fibroblast growth factor, and platelet-derived growth factor aid in the formation of granulation tissue and neovascularization. The amnion has ability to form early physiologic "seal" with the host tissue and decrease the host immunologic response through mechanisms such as localized suppression of polymorphonuclear cell migration.[7]

Thus, in the present study, a comparative evaluation was done to compare the root coverage obtained on areas of isolated gingival recession by using CAF along with PRF as well as placental membrane allograft (amnion) in the treatment of gingival recession.

2. Materials and Methods

The present study was carried out on 30 selected sites having either Miller's Class I or II marginal tissue recession in patients recruited from the outpatient Department of Periodontology at Buddha Institute of Dental Sciences and Hospital, Post Graduate Institute and Research Centre, Patna, Bihar. This is a clinical study designed as a split mouth randomized controlled trial. Each site constitutes one sample of the study. A prior approval of the institutional ethical committee was taken to conduct the study.

2.1. Inclusion criteria

Systemically healthy patients with Miller's class I and class II gingival recessions with no radiographic evidence of interdental bone loss were selected for the study.

2.2. Exclusion criteria

1. Patients with known systemic diseases or immune deficiency
2. Patients under medication that are known to affect periodontal healing
3. Patients with psychiatric disorder, pregnancy and lactating mothers
4. Patients with a habit of tobacco use in any form
5. Patients found to be incompetent in maintaining oral hygiene
6. Patients with inability or unwillingness to complete the trial and who were participating in another clinical trial.
7. 30 Amniotic membranes (freeze dried irradiated) were procured from Tata Memorial Hospital, Tissue Bank, Mumbai.(Figure 1).

Fig. 1: Amnion membrane used for root coverage procedure

2.3. Preparation of PRF membrane

Preoperatively, 10 ml of intravenous blood was collected in a syringe, later transferred into a test tube and centrifuged immediately without the addition of anticoagulant at 3000 revolutions per minute for 10 minutes. The absence of anticoagulants initiates the activation of platelets when they come in contact with the walls of the test tube. The resultant product consists of the three layers–top most layer consisting of acellular plasma, PRF clot in the middle, red blood cells at the bottom [Figure 2]. The PRF clot was separated from the other two layers and PRF was obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.[Figure 3].

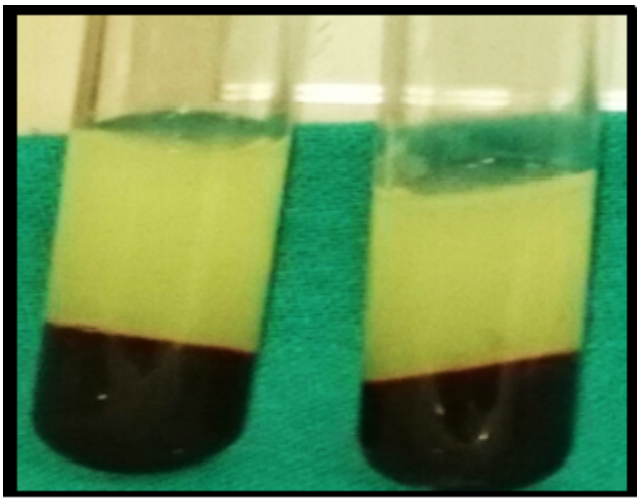

Fig. 2: Centrifuged blood sample

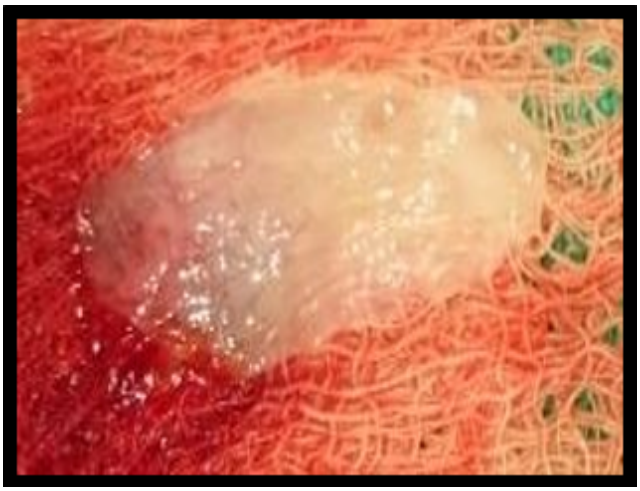

Fig. 3: Platelet-rich fibrin membrane

15 sites were randomly assigned for the test group (CAF with Amnion membrane) and another 15 sites were assigned for the control group (CAF with placental membrane allograft -amnion). All subjects received clinical periodontal examination by a single examiner.

Recession depth (RD) was measured at the midfacial region of the tooth from CEJ to the free gingival margin with UNC-15 periodontal probe at baseline and compared at 1, 3 and 6 months post–operatively between test and control sites. Acrylic stent was made for standardization of the study.

Fig. 4: Pre-operative view

2.4. Examiner calibration

Eight non-study patients with gingival recession were recruited for calibration. The single designated examiner recorded Recession Depth in each patient. The same examiner repeated the procedure after 24 hours. The intra examiner error was determined based on repeated RD measurement. The resultant correlation coefficient was 0.94 ± 1 mm and was accepted to proceed for the study.

2.5. Surgical procedure

Informed consent was taken from all subjects recruited for the study.

In group 1 patients just before surgery, intravenous blood was collected in a 10-ml test tube and a PRF membrane was prepared. Standard PRF was prepared according to Choukroun's PRF protocol. The surgical site was anesthetized by using 2% xylocaine with adrenaline (1:800000). CAF was performed by making two horizontal incisions with respect to the distal and mesial interdental papillae of the surgical sites, followed by a crevicular incision. Then two vertical releasing incisions at the mesial & distal aspects of the surgical site were given (Figure 5). Full thickness flap followed by a partial thickness flap

was reflected. After reflection of the flap, the previously prepared PRF membrane was placed on the recession defects, immediately below the CEJ. (Figure 6). The flap was coronally advanced and sutured.

Fig. 5: Sulcular and vertical incision

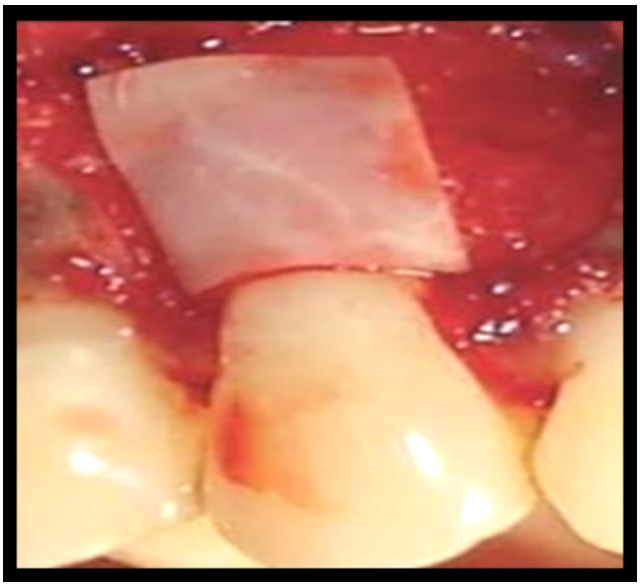

Fig. 6: PRF membrane placed

For group 2 patients, the surgical preparation was similar to the control sites and the amnion membrane was placed over the denuded root surface just below the CEJ (Figure 7) and the flap was sutured (Figure 8).Recession depth was recorded at 1, 3, and 6 months post - operatively and were compared with the baseline data.

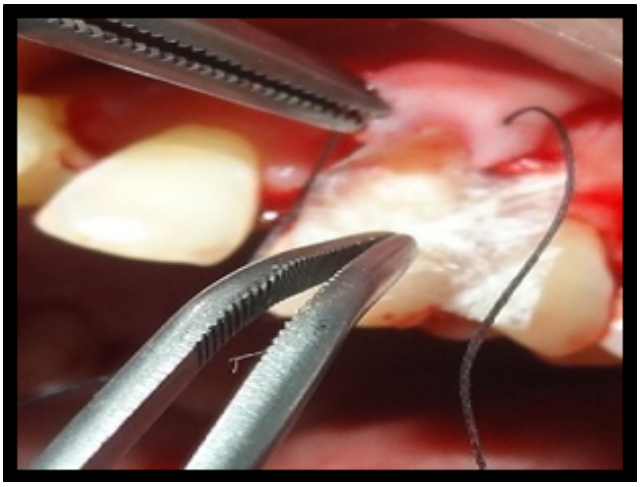

Fig. 7: Amnion membrane placed

Fig. 8: Suture placed

3. Results

Results obtained in both the groups were compared at baseline, and at one month, three months and six months after the procedure.

The findings were tabulated and statistically analyzed. Data obtained was compiled on a MS Office Excel Sheet (v 2010, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using the Statistical package for social sciences (SPSS v 21.0, IBM). Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data have been depicted. Normality of numerical data was checked using Shapiro-Wilk test & was found that the data did not follow a normal curve; hence non-parametric tests have been used for comparisons. Inter group comparison (2 groups) was done using Mann Whitney U test. Intra group comparison was done using Friedman's (for >2

observations) followed by pairwise comparison using Wilcoxon Signed rank test. Comparison of frequencies of categories of variables with groups was done using chi square test.

Tables 1, 2 and 3 depicts distribution of recession depth as per site, sex and arch.

3.1. Frequency tables

Table 1: Distribution as per Site

13	4	20.0
14	1	5.0
15	2	10.0
22	1	5.0
23	5	25.0
32	2	10.0
42	3	15.0
Total	20	100.0

Table 2: Distribution as persex

	Frequency	**Percent**
F	8	40.0
M	12	60.0
Total	20	100.0

Table 3: Distribution as perarch

	Frequency	**Percent**
Man	5	25.0
Max	15	75.0
Total	20	100.0

Table 4: Comparison of frequencies of categorical variables vs groups

A: Site Group

		Group 1	2	Total	Chi square value	p value of chi square test
Site	12	1	1	2		
	13	2	2	4		
	14	1	0	1		
	15	1	1	2		
	22	0	1	1	2.533	0.925#
	23	3	2	5		
	32	1	1	2		
	42	1	2	3		
	Total	10	10	20		

There was a statistically non- significant difference seen for the frequencies between the groups (p>0.05).

B: Sex Group

		Group 1	2	Total	Chi square value	p value of chi square test
Sex	F	4	4	8		
	M	6	6	12	0.000	1.000
Total		10	10	20		

There was a statistically non-significant difference seen for the frequencies between the groups (p>0.05)

C: Arch Group

		Group 1	2	Total	Chi square value	p value of chi square test
Arch	Man	2	3	5		
	Max	8	7	15	0.267	0.606#
	Total	10	10	20		

There was a statistically non-significant difference seen for the frequencies between the groups (p>0.05).

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Table 4, A, B and C) depicts comparison of frequencies of categorical variables vs group (site, sex and arch. There was a statistically non-significant difference seen for the frequencies between the groups (p>0.05).

4. Discussion

CAF with Subepithelial connective tissue graft has been shown to be a predictable procedure to treat Miller's class I and class II mucogingival recession. Subepithelial connective tissue graft is often used in combination with coronally advanced flap for root coverage. This combination has shown high success and predictability rates.[8]

But sub epithelial grafts have several adverse effects such as discomfort with or without pain associated with a donor site's source of the connective tissue grafts and the second wound area. Hence, there is a search for an alternative to connective tissue graft which would render better results than the coronally advanced flap procedure alone in case of treatment of Miller's class I and class II recession defects.[9] HAM has been tried by some periodontists but the literature regarding HAM is mostly in the form of case reports *Rucha Shah 2014, Shetty et al 2014, Anamika Sharma and Komal Yadav 2015). Therefore,* this present RCT was conducted to see the additional benefits of using HAM, if any, along with the standard procedure of CAF.

Table 5: Inter group comparison of recession depth (n=10 per group)

Group		Mean	Std. Deviation	Median	Mann-Whitney U value	Z value	p value
Baseline	1	2.70	.949	2.5	41.500	-0.713	0.476#
	2	2.80	.632	3			
1 month	1	2.150	1.7005	1.25	48.000	-0.156	0.876#
	2	2.250	1.4767	2.25			
3 months	1	2.450	1.9214	1.75	45.500	-0.350	0.727#
	2	2.450	1.3427	2.25			
6 months	1	1.750	1.7200	1	43.500	-0.499	0.618#
	2	2.050	1.4991	2.25			

There was a statistically non-significant difference seen for the values between the groups (p>0.05) For RD at all time intervals

Table 6: Intra group comparison of RD for group 1

Time	Mean	Std. Deviation	Minimum	Maximum	Median	Mean rank	Chi square value	p value of Friedman Test
Baseline	2.70	.949	2	5	2.50	3.15	8.864	0.031*
1 month	2.150	1.7005	.0	5.0	1.250	2.35		
3 months	2.450	1.9214	1.0	7.0	1.750	2.70		
6 months	1.750	1.7200	.0	5.0	1.000	1.80		

There was a statistically significant / highly significant difference seen for the values between the time intervals (p<0.01, 0.05) with higher values at baseline and least at 6 months.

Table 7: Pairwise comparison

Time intervals	Z value	p value of WSR test
1 Month – Baseline	-1.318	0.187#
3 Months – Baseline	-0.690	0.490#
6 Months – Baseline	-2.154	0.031*
3 Months - 1 Month	-1.134	0.257#
6 Months - 1 Month	-1.633	0.102#
6 Months - 3 Months	-2.070	0.038*

There was a statistically significant / highly significant difference seen for the values between the time intervals (p<0.01, 0.05) between Baseline vs 6 months, 3 months vs 6 months While there was a statistically non-significant difference seen for the values (p>0.05) between all other time intervals

Table 8: Intra group comparison of RD for group 2

Time	Mean	Std. Deviation	Minimum	Maximum	Median	Mean rank	Chi square value	p value of Friedman Test
Baseline	2.80	.632	2	4	3.00	3.10	6.600	0.086#
1 Month	2.250	1.4767	.0	4.0	2.250	2.30		
3 Months	2.450	1.3427	1.0	5.0	2.250	2.60		
6 Months	2.050	1.4991	.0	4.0	2.250	2.00		

There was a statistically non-significant difference seen for the values between the time intervals (p>0.05)

Table 9: Pairwise comparison

Time intervals	Z value	p value of WSR test
1 Month – Baseline	-1.318	0.187#
3 Months – Baseline	-1.052	0.293#
6 Months – Baseline	-1.807	0.071#
3 Months - 1 Month	-1.000	0.317#
6 Months - 1 Month	-1.414	0.157#
6 Months - 3 Months	-1.633	0.102#

There was a statistically non-significant difference seen for the values (p>0.05) between all time intervals.

The present randomized controlled trial has shown that the result of recession coverage with CAF with PRF is significantly better than that with CAF + AM. Comparable results have also been achieved with the use of CAF + AM or amnion chorion membrane by *Lafzi et al 2016, Sarah Grace George 2017, Dr. Vinesh Komanth 2017, Md. Rehan 2018.*

The reason for limited success of HAM in the treatment of soft tissue defects could not be found in the literature.

When HAM is used as an adjunct to CAF in the treatment of recession defects, the presence of additional material in the form of HAM makes it difficult to manipulate soft tissue, whereby primary flap closure requires stretching the tissue to a great extent, rendering it difficult to suture the flap in a tension free fashion. This might be one of the reasons for the less than optimal results achieved by HAM in soft tissue augmentation. Additionally stretching the tissue in the esthetic zone might change the tissue quality.In case the facial flap requires a lot of stretching the vestibular tissue is pulled coronally resulting in a thin biotype and insufficient volume of attached tissue. Equally if the tissue is pulled too taut in patients with thick tissue, it stretches thin and becomes prone to rupture.[10] It may be because of the same reasons that the amount of root coverage obtained with CAF is greater than that observed with CAF+GTR, although the use of GTR results in significantly greater alveolar crest level gain.[7] Comparable results have also been shown with the adjunctive use of acellular dermal matrix with CAF, due mainly to the aforesaid reasons.[11]

It has also been noted during and after the surgical procedures that HAM undergoes some shrinkage with time, which has the potential to create a dead space between the root surface and the tissues, which might invite microorganisms and jeopardize the healing process. No mention of this observation has been noted in the literature.

The addition of PRF as a membrane to CAF showed an increase in width of keratinized gingiva and a decrease in clinical attachment loss, recession depth, which was statistically significant at 6 months. This in accordance with study done by Jankovic et al., who evaluated and compared the clinical effectiveness of platelet rich plasma and connective tissue graft in the treatment of gingival recession.[12]

The present study has some limitations. Firstly it was not blinded at any stage. A single operator performed all the surgical procedures and it was the same person who examined, monitored and maintained all the patients at all stages of the study which may have contributed to observer bias.

Secondly, the sample size of the present study was small and the duration of the study was only for 6 months. The AM used for the present study, procedure from Tata Memorial Hospital Tissue Bank, was freeze dried and irradiated which is not available commercially, so its accessibility is limited and these processing methods might have led to deterioration of some of the properties of the material. So HAM preserved by other techniques like cryopreservation may also be used in future studies for appraisal of the full spectrum of benefits of this material.

Moreover, atraumatic needles and suture materials, which are ideal for mucogingival procedures could not be used for this study. 4'-0 silk sutures and 3/8 circle stainless steel needles have been used for all the cases.

It has also to be noted that recession coverage correlates inversely with initial recession depth, indicating that deeper defect would benefit more with root coverage procedures.[13]

The secretion profile of three isoforms of cytokines [platelet-derived growth factor-BB (PDGF-BB), transforming growth factor β-1 (TGFβ-1) and insulin growth factor-1(IGF-1)] within the different parts of the PRF collection tube and a whole range of concentrated platelet rich plasma were studied. From their comparative biochemical analysis, authors came to the conclusion that PRF consists of an intrinsic assembly of cytokines, glycemic chains, and structural glycoproteins enmeshed in a slowly polymerized fibrin network having synergistic effects on healing processes-1) and insulin growth factor-1(IGF-1)] within the different parts of the PRF collection tube and a whole range of concentrated platelet rich plasma were studied. From their comparative biochemical analysis, authors came to the conclusion that PRF consists of an intrinsic assembly of cytokines, glycemic chains, and structural glycoproteins enmeshed in a slowly polymerized fibrin network having synergistic effects on healing processes.[14] Therefore, PRF can be considered as not only a new generation of platelet gel but a completely usable healing concentrate gel. They also evaluated the quantity of five significant cell mediators within PRP supernatant and PRF clot exudates, three pro- inflammatory cytokines (IL-1β, IL-6, TNF-α), and anti-inflammatory cytokine (IL-4), vascular endothelial growth factor (VEGF) by collecting blood from fifteen 20-28 year old healthy non-smoker males and stated that the cytokine concentrations were high in PRF clot exudates than in plasma and serum samples with the exception that the concentration of VEGF is significantly high in serum samples.[15]

The most reliable outcome variable for assessing periodontal regeneration is human histology.13 Due to ethical considerations and patient's management limitations, no histological evaluation was performed in the present study; therefore the effect of HAM on overall regenerative capacity remains to be determined.

It is necessary to look further into platelet and inflammatory features of PRF. Only a perfect understanding of its components and their significance will enable us to comprehend the clinical results obtained and subsequently extend the fields of therapeutic application of this protocol.

5. Source of Funding

None.

6. Conflict of Interest

None.

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