Original Article

Comparative Assessment Of Platelet Rich Fibrin Placed Through Tunnel And Pouch Technique With And Without The Use Of Enamel Matrix Derivatives For Recession Coverage – A 12 Month Randomized Control Trial

Ashish Bali, Sandeep Kumar Dubey, Pritish Chandra Pal, Iqbal Singh

Abstract:

Introduction: Apical migration of gingival margin i.e. gingival recession is one of the most common periodontal esthetic issues. Successful minimally invasive management of gingival tissue recession is still a concern in esthetic dentistry.

Material & Method: 50 sites in 50 patients with Miller' class I/II recessions were treated with tunnel and pouch technique (TPT) and platelet rich fibrin (PRF) with (Test group, n=25) or without (Control group, n=25) enamel matrix derivative (EMD) application. Subjects were followed for 12 months. Gingival recession depth (RD), Clinical attachment level (CAL), percentage of root coverage, Gingival Index, Plaque Index were measured at baseline and at 12 month. Data obtained were fed to IBM SPSS 22.0. Wilcoxon Sign Rank Test for Intragroup statistical analysis and Student't' test for intergroup statistical analysis were performed. The level of significance was fixed at $p \leq 0.05$.

Results: CAL in control group was 2.60±0.91mm and in test group it was 3.33±1.05mm at 12th month. The mean difference of CAL gain was 0.73±0.14mm, which was statistically significant (p=0.025). At 12th month the mean amount of RD coverage in control group was 2.13±0.74mm and in the test group it was 2.67±0.72mm. The mean RD difference of 0.54±0.02mm was found to be statistically highly significant (p=0.012).

Conclusion: TPT technique when combined with PRF and root bio modification with EMD provides convincing results in miller's class I or II gingival soft tissue recession cases.

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Introduction:

Apically shifted marginal gingiva from its natural location i.e. cemento-enamel junction (CEJ) is termed as gingival tissue recession (GR).[1] Numerous developmental, anatomical or acquired etiological factors like abnormal tooth position, frenal pull, periodontal disease, bony fenestration or dehiscence, faulty tooth brushing, iatrogenic factors may lead to root exposure. Loss of gingival tissue and subsequent root exposure leads to unaesthetic appearance, dentinal hypersensitivity and root caries.[2] Successful root coverage has turned into a crucial therapeutic concern due to escalated cosmetic and functional demands in contemporary society. The desired hope of recession coverage intends to achieve complete root coverage (CRC) to provide a pleasing esthetic appearance. Periodontal plastic surgeons have assorted diverse surgical strategies to cover the pathologically denuded tooth surfaces. Conventional coronally displaced flap, rotational or advanced pedicle procedure, soft tissue grafting (free gingival or connective tissue) and modification of conventional methods such as with alternative soft tissue grafting, resorbable or non-resorbable membranes have been applied as evident from literature.[3] Reports have demonstrated that advancing the gingival flap through tunnel and pouch technique (TPT) has specific promising esthetic results and therefore classically employed. TPT is a reliable formulaic procedure in the treatment of Miller Class I or II GRs.[4,5] It has also been used with many regenerative materials like the guided tissue membranes, enamel protein derivatives, tissue engineered human fibroblast, alloderm material, dermal substitutes, platelet rich fibrin (PRF), placental

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Keywords

Gingival recession, root coverage, PRF, EMD

membranes with good reported clinical outcomes. Since the 1950s, several mucogingival surgical techniques aiming for CRC have been published with conflicting rates of success and predictability.[6] Concentrated growth factors are intricate in complex healing activity and PRF are assumed as key proponent of tissue regeneration. It contains platelet derived growth factors (PDGF), transforming growth factors (TGF) and diverse unidentified growth factors. Periodontalligament cells of human contain major mitogens PDGF. Type I collagen an obligatory element ofhealing is also stimulated by the PRF derived fibrin clot. Though autogenous soft tissue grafting is believed as a benchmark treatment for root coverage, alternate use of PRF eliminate the need for a donor site, minimize postsurgical hassle, and nurture expeditious gingival healing making minimally invasive approach.[7]

Heijl et al.[8] reported periodontal regeneration in an experimental recession defects with enamel matrix derivative (EMD). Former studies showed that EMD application on recessed root exterior imitate cementogenesis of nascent root development. Mellonig [9]histologically confirmed new cementum formation with EMD. Rasperini et al[10]explained a new attachment in GR treated with autogenous tissue graft and EMD. Hinged upon these former evidence, it seems equitable to use EMD for ameliorating the clinical outcomes of GR defects. As per our knowledge the literature is also scattered regarding this aspect. Hence, comparing the TPT and PRF combination with and without EMD application for the treatment of GR would be a minimally invasive and novel approach. Despite the availability of myriads of material and technique for treatment of GR, there is no authentic lucidity on the efficacy of these procedures.

Therefore this current research was done to clinically appraise and compare the efficacy of PRF placed through TPT with and without the use of EMD in the treatment of Miller's Class I or II GR sites and followed up for 12 months.

Material and methods:

Overall 50 patients with age group between 18-60 years (mean age 38.5) with Miller's class I /II GR present between 2nd premolars (upper/lower) teeth requiring surgical root coverage were selected from the out-patient department. 50 GR sites were randomly allocated into 2 groups by ablinded researcher. Group A (n=25) included – CAF with PRF and group B (n=25) included – EMD application in co-occurrence with TPT and PRF.

The Institutional Ethical Board approved the study protocol and consent form of the current randomized controlled trial. Each study participants were given a detailed verbal and written description of the proposed study, benefit and possible risk factors associated with the surgical intervention and anticipated outcome of the treatment. Those who agreed voluntarily and meet the inclusion criteria of the study signed a consent form.

Study participants were in general good health. Inclusion criteria for the study were: patients exhibiting Miller's Class I/II GR defect[5] in maxillary or mandibular teeth (between 2nd premolars); age between 18-60 years; no relevant systemic disease; presence of identifiable CEJ; no radiographic evidence of interdental bone loss; no occlusal interferences, caries or restoration on the tooth of interest.

Exclusion criteria were – patient under medication known to interfere with gingival health or healing; habitual smoker or tobacco chewer; pregnant and lactating mothers; malposed/rotated teeth; history of periodontal surgery at investigation site within 6 months.

Initial Therapy: Preliminarily study participants received a complete scaling and root planing. If the teeth of interest had any distinguished root convexities that were planned to reduce. A punctilious plaque control programme was initiated for each study subjects 3 weeks before surgical procedure including stipulation in suitable brushing technique with soft tooth brush.

Surgical protocol:

Antiseptic solution (Betadine 10%, Skincare Surgi Pharma Pvt Ltd, Mumbai, Maharashtra, India) was applied on the field of operation with cotton swab. Surgical site was anesthetized using 2% xylocaine with 1:50,000 epinephrine (Lignox 2%, ICPA, Mumbai, India) on both the facial and lingual aspect.

Total of 25 sites in 25 patients were treated in the control group. The procedure started with a labial crevicular incision bard parker (BP) blade No.12 around the involved tooth except the interdental papilla region. This was followed by tunnel preparation by using tunneling instrument apically surpassing the mucogingival junction (MGJ) and proximally on both side of the defect extending around the abutting teeth. Adequate mobility of the flap was ensured to allow for flap advancement without tension. Root bio-modification of the exposed root surface was done by applying 24% EDTA gel (Prevest EDTA Gel 24%, Prevest Dentpro, Jammu, India) for 2 minutes and then washed with saline. 10 ml of venous blood was collected in 2 glass tubes from antecubital fossa using standardized protocol. Glass test tubes were then placed in a centrifugal machine (Labcare Ltd., Haryana, India) and centrifuged at 3000 revolutions/minute for 10 minutes. Blood settled into the following layers: red lower fraction containing red blood cells,

upper straw colored cellular plasma and the middle part containing the fibrin clot. The middle fraction was collected and membrane was made using PRF box (GDC Ltd., Punjab, India). PRF membrane was passed through the tunnel and sutured (Ethicon vicryl sutures USP 4-0, USA). Periodontal dressings were placed over it.

Total of 25 sites in 25 patients were treated in the test group. TPT design and PRF placement

was identical to the one described in the control group, except for the addition of Emdogain. After flap preparation the exposed root surfaces were conditioned with a 24% Prefgelfor 2 minutes in order to remove the smear layer. The area was thoroughly rinsed with saline and dried. Commercially available EMD (Emdogain gel) was applied to the exposed root surface followed by PRF placement. Suturing was done as described in control group.

Sutures were removed at 14th day. Further evaluation was done at each month. Plaque control initiatives were followed till the study period.

Clinical measurements:

Following measurements were taken using CEJ as a fixed point at baseline and at 12th month

1. Recession depth (RD) - CEJ to gingival margin
2. Clinical attachment level (CAL) - CEJ to base of gingival sulcus

Additionally the percentage of root coverage was calculated after 12 months according to thefollowing formula: [(Preoperative RD – Postoperative RD) X 100] ÷ (Preoperative RD)

Also, gingival inflammation was monitored by using Gingival Index (Loe and Silness 1963).[11]Oral hygiene effectiveness was monitored by using Plaque Index (Silness and Loe 1964).[12]

Statistical analysis: Statistical analysis was done using IBM SPSS Software Package 22.0 (IBM Tech. Corp, New York, USA). Descriptive statistics were expressed as mean and standard deviation (SD). Data were analyzed using Wilcoxon Sign Rank Test for Intragroup statistical analysis (from baseline to 12th months in each group) and Student't' Test for intergroup statistical analysis (between test and control group). The level of significance was fixed at p≤ 0.05.

Results:

Control group:

CAL: CAL was compared pre and post-operatively over 12 months period. The mean depth of CAL at baseline was 4.67±0.72 mm which was reduced to 2.07±0.80mm at 12 month. The mean difference of 2.60±0.91mm was found to be statistically highly significance (p=0.000). [Table 1]

RD: Mean RD at baseline was 3.00±0.76mm which was reduced to 0.87±0.64mm at 12 month. The mean RD difference compared to baseline was 2.13±0.74 mm which was found to be statistically highly significant (p=0.000). [Table 1]

PI: The mean and standard deviation value for PI at baseline and 12 months was 0.16 (± 0.28) and 0.26 (± 0.25) respectively & showed no significant difference in baseline to 12 month interval (F value = 1.068, p=0.349). [Table 1]

GI: The mean and standard deviation value for PI at baseline and 12 months was 0.32 (± 0.47) and 0.40 (± 0.50) respectively & showed no significant difference in baseline to 12 month interval (F value = 0.220, p =0.803). [Table 1]

Test group:

CAL: The mean depth of CAL at baseline was 4.87±0.83mm which was reduced to 1.53±0.74mm at 12th month. The mean difference of 3.33±1.05mm was found to be statistically highly significant (p=0.000). [Table 1]

RD: The mean RD at baseline was 3.02±0.76mm which was reduced to 0.33±0.49mm at 12th month and mean difference compared to baseline was 2.67±0.72mm which was found to be statistically highly significant (p=0.000). [Table 1]

PI: The mean and standard deviation value for PI at baseline and 12 months was 0.16 (± 0.22) and 0.16 (± 0.23) respectively & showed no significant difference in baseline to 12 month interval (F value = 0.000, p=1.00). [Table 1]

GI: The mean and standard deviation value for PI at baseline and 12 months was 0.32 (± 0.47) and 0.40 (± 0.57) respectively & showed no significant difference in baseline and 12 month interval (F value = 0.364, p= 0.696). [Table 1]

Inter group comparison:

CAL:

At 12th month the mean CAL gain in control group was 2.60±0.91mm and in test group it was 3.33±1.05mm. The mean difference of CAL gain was 0.73±0.14mm, which was statistically significant (p=0.025). [Table 2]

RD:

At 12th month the mean amount of RD coverage i.e. root coverage in control group was 2.13±0.74mm and in the test group it was 2.67±0.72mm. The mean RD difference of 0.54±0.02mm was found to be statistically highly significant (p=0.012). [Table 2]

Discussion:

Periodontal therapy has been directed primarily at elimination of periodontal disease and maintaining the function and health of the dentition. However it has become increasingly focusedon esthetics, which extends from hard and soft tissue management to tissue augmentation. Investigation of etiologic factors and consideration of therapeutic options directed at CRC aid in achieving an esthetic and natural appearance of the newly gained tissue. Patient's esthetic demands, progressive root surface exposure and dental hypersensitivity are the main indication for root coverage procedures. Among the various plastic procedures performed to relieve exposed root surfaces, TPT technique have shown more predictable recession coverage with apparently satisfactory esthetic results. Nevertheless, TPT when used alone is unstable on long-term, in spite of having the advantage of low morbidity. Furthermore, it does not always result inthe regeneration of attachment apparatus which is a major risk factor in recurrence of GR. Therefore, TPT have been frequently combined with various regenerative materials aiming at attaining both regeneration of functional attachment apparatus and root coverage.[4,13]

Limitations like donor surgical site, technique

sensitivity, patient morbidity associated with procurement of autogenous gingival grafts led to the newer advancements as introduction of biomimetic agents such as platelet rich fibrin, bone morphogenic protein have given new promises for recession treatment. There has been an increasing interest in platelet concentrates in the field of periodontal regeneration. PRF developed by Choukroun et al. consistsof a fibrin polymer matrix accumulate platelets and released cytokines in a fibrin clot.[14] Slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines, circulating stem cells and glycemic chains in the fibrin meshes. It is also found that PRF organizes as a dense fibrin scaffold with a high number of leukocytes concentrate, slow release growth factors and glycoproteins. The release of these growth factors like transforming growth factor beta (TGF-β), platelet derived growth factor, and vascular endothelial growth factor (VEGF) stimulates cell migration and proliferation within the fibrin matrix. VEGF functions to start angiogenesis and cell growth. TGF-β is an inflammatory regulator can induce a massive synthesis of collagen and fibronectin.[15]

In our knowledge as per present literature none of the study has compared TPT+PRF combination with root bio modification using EMD and evaluated till 12 months long period. Therefore we can take reference of various closely similar studies to compare our result. Jankovic S et al. in 2012 in a 6 months randomized controlled trial found that PRF membrane provided clinically acceptable results and enhanced wound healing.[16] Present result is in accordance with the study performed by, Reddy S et al. in 2013[17 who also reported two cases where PRF membrane was used but in addition to coronally advanced flap technique and showed enhanced root coverage with increase in thickness of gingiva. Padma R et al. in 2013[18] found that addition of PRF to CAF technique provided superior root coverage. Eren G and Atilla G in 2014[19] reported that PRF can be an alternative to CTG membrane. Tunali M et al.[20] have shown CRC with both PRF and CTG membranes in 44 gingival recessions. Shiv Kumar et al.[21] obtained CRC at 73.86% of the sites treated with CAF + PRF procedure but 67.52% of root coverage at the CAF sites. These results might be due to the property of the PRF to progressively release cytokines and growth factors during fibrin matrix remodeling in the process of soft tissue healing. Salem S et al in 2020 evaluate the four-year outcomes of the CAF versus the pouch and tunnel (POT) technique, both combined with connective tissue graft. The POT technique allows for long-term clinical coverage of gingival recessions.[22] Vatsala Chandra and colleagues in 2022 reported root coverage of 73.75% ± 7.80% and 70.83% ±8.26% by using TPT with PRF andCTG. He also reported less discomfort and better esthetics outcome with TPT.[23] Inasu S. and Thomas B. in 2021 in a published case series showed goof clinical outcome while using TPT with PRF membrane.[24]

EMD mimics the function of enamel protein secreted by the inner layer of Hertwig's epithelial root sheath on the surface of new dentin. The material consists of matrix derived protein, primarily amelogenin, which is harvested from embryonic porcine teeth and studied in animals and humans providing evidence of tissue regeneration. Split mouth or controlled studies showed increased percentage of root coverage in defects treated with the TPT+EMD. The observed percentage of root coverage in test group is in accordance with the values in studies reported after the use of EMD, ranging from 80.8% to 93.8% at 6 months of follow up. This result is comparable to the results of studies done by Modica et al[25], Pizzo et al[26], Cueva et al[27], Castellanos et al[28]. Although EMD has been extensively used clinically but limited information is available regarding cell attachment or the mechanism of effect promoted by EMD. A recent study by Suzuki & Ohyama[29] provided the first evidence indicating that a bone sialoprotein like molecule in EMD and integrin on the surface of human periodontal ligament cell (HPDL) may mediate binding of EMD to the HPDL cell surface. The author also suggested that this might be crucial to the differentiation of these cells. This might explain why EMD was able to enhance the outcome of root coverage procedure utilizing TPT in the test group. [25,30] Role of root bio modification in success of root coverage has been found positive in our present study. Though root planning was performed in both the test and control group, additional EMD application was beneficial. Also patient compliance and participation was better as it was explained as minimally invasive method. TPT is less invasive than many conventional CAF procedure.

Conclusion: Though in recent years the role of root bio modification was questioned, this present study showed the use of EMD has significantly improved the root coverage percentage ascompared to control group. Also the use of TPT and PRF is proved to be a minimally invasive root coverage procedure. The degree of recession, root concavities, patients personal and oral hygiene habits may play a significant role in success or failure of root coverage.

Table - 1 : Intra group comparison from baseline to 12th month of RD and CAL in control and test group

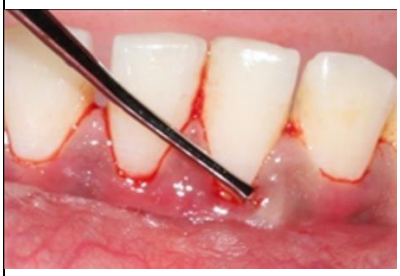
Group	Parameters	Baseline		12 month after surgery						
		Mean	±SD	Mean	±SD	Mean diff	±SD	't' value	'p' value	Sig/NS
Test	RD	3.02	0.76	0.33	0.49	2.67	0.72	11.47	0.000	HS
Test	CAL	4.87	0.83	1.53	0.74	3.33	1.05	11.55	0.000	HS
Control	RD	3.00	0.76	0.87	0.64	2.13	0.74	8.34	0.000	HS
Control	CAL	4.67	0.72	2.07	0.80	2.60	0.91	9.34	0.000	HS

Table - 2 : Inter group comparison of RD coverage and CAL gain among control and test group at 12th month clinical measurement

S. No.	Parameters	N	Mean	SD	Mean Diff	SD	't' value	'p' value	Sig/NS
1	RD Coverage	Control	2.13	0.74	0.54	0.02	1.99	0.012	HS
		Test	2.67	0.72					
2	CAL Gain	Control	2.60	0.91	0.73	0.14	2.04	0.025	Sig
		Test	3.33	1.05					

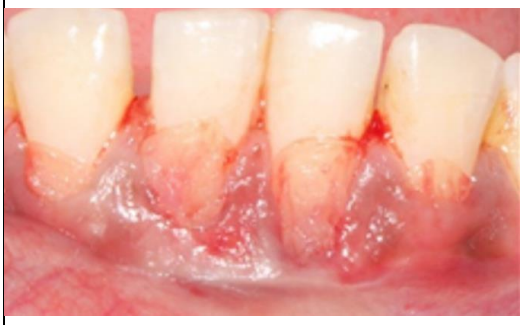
Control group

Pre-operative view of gingival recession



Sulcular incision followed by tunnel preparation by using tunneling instrument

Adequate tunnel preparation to move the gingival unit coronally



Platelet rich fibrin membrane placement to the recession site through tunnel

Test group

Suturing done and flap is coronally repositioned with suture and composite

12 month follow-up showing complete recession coverage

Pre-operative view of gingival recession

Sulcular incision followed by tunnel preparation by using tunneling instrument

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Suturing done and flap is coronally repositioned with suture and composite

12 month follow-up showing complete recession coverage

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