Comparison of two surgical techniques in the treatment of multiple gingival recessions sandwiched with a combination of A-PRF and L-PRF

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Abstract  Aim: The aim of this study is to compare semilunar vestibular incision technique with pouch and tunnel technique in combination with A-PRF and L-PRF for treatment of Miller’s class I and II multiple gingival recessions.

Method: This is a randomized, controlled, double-blinded, split mouth study which consists of 16 systemically healthy patients with 96 sites and a mean age of 34.2 years, and divided randomly into 2 groups, Group A consists of semilunar vestibular incision technique sandwiched with A-PRF and L-PRF and Group B consists of Pouch and tunnel technique sandwiched with A-PRF and L-PRF. Clinical parameters were recorded at baseline, 3 months and 6 months which include plaque index, gingival index, recession depth, recession width, clinical attachment loss and width of keratinized tissue.

Results: All the clinical parameters showed significantly better levels for both the groups from baseline to 6 months. Semilunar vestibular incision technique showed greater significance when compared to pouch and tunnel technique from baseline to 6 months post operatively. Semilunar vestibular incision technique showed promising results than pouch and tunnel technique for the treatment of multiple gingival recessions.

Conclusion: The combination of A-PRF and L-PRF with pouch and tunnel technique and semilunar vestibular technique showed better outcome 6 months post operatively. Semilunar vestibular incision technique showed promising results than pouch and tunnel technique for the treatment of multiple gingival recessions.

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1. Introduction

The displacement of the gingival margin apical to the cemento-enamel junction CEJ with the loss of periodontal connective tissue fibers along with root cementum and alveolar bone is defined as gingival recession (Wennstrom et al., 2008). Indications for root coverage procedures are esthetic demands, cervical abrasions, root hypersensitivity and root caries lesions (Goldstein et al., 1996).

Various procedures for the treatment of multiple gingival recessions include coronally advanced flap with/without mucosal grafts, sub epithelial connective tissue graft (SCTG) and guided tissue regeneration. Allen in 1994 modified Raetzke’s technique and described it as “Tunnel or supraperiosteal envelope technique,” for treatment of adjacent multiple gingival recessions (Allen, 1994). Zabalegui and others treated multiple gingival recessions by creating a tunnel under the areas of gingival recession to receive the connective tissue graft thus avoiding dissecting the intermediate papilla and improving blood supply to the flap (Zabalegui et al., 1999). Mahn adapted the tunnel approach for acellular dermal connective tissue grafting by using full thickness procedure with vertical incisions (Tozum and Dini, 2003). Although there are numerous techniques available, some techniques when attempted produce unsatisfactory results. The reasons could be; improper case selection, improper technique selection, inadequate root preparation, insufficient height of interdental bone and soft tissue, improper surgical technique, insufficient blood supply from the surrounding tissues due to inadequate recipient site preparation and flap penetration (Dani et al., 2014). The semilunar vestibular incision technique, which was described by Dr. P.D. Miller in a conference at Pune in 2011, describes a method of coverage of multiple recessions with the advantage of increasing the width of attached gingiva, enhancing the depth of the vestibule and avoidance of the second surgical site (Pandit et al., 2015).

Various adjunctive agents, such as recombinant human growth factors and platelet-rich concentrates (Jankovic et al., 2012; Agarwal et al., 2013) were used to accelerate healing and further enhance clinical outcomes. Many studies revealed that platelet concentrates for surgical use can be used as efficient adjuvants for tissue repair (Bielecki and Dohan Ehrenfest, 2012; Gruber et al., 2002; Carlson and Roach, 2002; Sanchez et al., 2003; Anitua et al., 2004). Leukocyte- platelet-rich fibrin (L-PRF) and Advanced platelet rich fibrin (A-PRF) are considered as autologous biomaterials (Choukroun et al., 2001). They represent a more recent generation of platelet concentrates. Unlike other platelet-rich products, these materials does not require an anticoagulant or bovine thrombin (Choukroun et al., 2006). It is simply centrifuged blood without any additives, which makes it possible to avoid all of the legal restrictions related to the re-implantation of blood-derived products.

This study is done to evaluate the advantages of a combination of L-PRF and A-PRF membranes with comparing pouch and tunnel technique and semilunar vestibular incision technique for treatment of multiple gingival recessions.

2. Materials and methods

The study population included patients who reported to the department of Periodontics, St. Joseph dental college, Eluru, between January 2016 to January 2017.

Sample size and randomization: Assuming 95% confidence interval and 80% power, 16 subjects (8 male and 8 female) were included in the study.
Systemically healthy subjects with esthetic complaints due to excessive tooth length, periodontally healthy subjects with multiple (>3 teeth) with Miller’s Class I and II recession defects (≥1 mm in depth) on adjacent teeth in the maxilla, presence of identifiable cementoenamel junction (CEJ) were included in the study.

A total of 96 sites in 16 patients with age range between 25 and 50 years mean age: 34.2 years were selected for the study. A randomized coin toss split mouth, double blinded study was undertaken to evaluate the efficiency of Semilunar vestibular incision technique vs. Pouch and tunnel technique with a combination of A-PRF and L-PRF in the treatment of Miller’s class I and II multiple gingival recessions. The selected sites were divided into Group A and Group B.

2.1. Group A

48 sites were treated with semilunar vestibular incision technique in combination with A-PRF and L-PRF.

2.2. Group B

48 sites were treated with pouch and tunnel technique in combination with A-PRF and L-PRF.

Approval of the study was obtained from the institutional ethical committee (CEC/16/2015-2016) and the study protocol was in accordance with the Declaration of Helsinki on experimentation involving human subjects. An informed consent was taken from all the participants before the commencement of the study. Patients with smoking, uncontrolled local or systemic diseases that might be a contraindication for periodontal surgery, pregnancy or lactating women, severe immune deficiency, coagulation defects, or on current anticoagulation treatment were excluded from the study.

Clinical parameters were recorded at baseline, 3 months and 6 months after surgery which include plaque index (PI), gingival index (GI), recession depth (RD), recession width (RW), width of keratinized tissue (KT) and clinical attachment loss (CAL). The measurements were taken using UNC-15 probe on the mid-buccal, disto-buccal, mesio-buccal, mid-lingual, mesio-lingual and disto-lingual aspects of the teeth and rounded to the nearest 0.5 mm.

**Primary and secondary outcome variables:** Primary outcome variables were the difference between groups for mean RD, RW, CAL and KT at baseline, 3 months and 6 months post-operatively. Secondary outcome variables included the mean levels of PI and GI at baseline, 3 months and 6 months post-operatively.

Intraexaminer Reproducibility: Five subjects, not related to the study and each showing two pairs of contralateral teeth (single- and multirooted) with RD >2 mm on at least one aspect of each tooth, were used to calibrate the examiner. The examiner evaluated the subjects on two occasions 48 hours apart. Calibration was accepted if 90% of the recordings could be reproduced within a 0.5 mm difference.

2.3. Presurgical protocol

The surgical treatment plan was explained to all the patients prior to the surgery. Oral prophylaxis was done and oral hygiene instructions with proper brushing technique were clearly explained to all the patients. All the patients were recalled 15 days after oral prophylaxis for surgical procedure.

2.4. Surgical technique

2.4.1. L-PRF and A-PRF procedure

L-PRF and A-PRF was prepared according to the protocol developed by Choukroun et al. (2001). Immediately prior to surgery, 10 mL of blood was drawn from each patient by venipuncture of the antecubital vein and is collected in two sterile glass test tubes, each with 5 mL of blood, without any anticoagulant. The blood was quickly collected, and the tubes were immediately centrifuged. For L-PRF, the tubes were centrifuged at 2700 rpm for 12 min and for A-PRF, 1500 rpm for 14 min at room temperature. After centrifugation, the PRF clots were removed from the tubes using sterile tweezers and placed on sterile woven gauze. Both L-PRF (Fig. 1A) and A-PRF (Fig. 1B) membranes were prepared by compressing clots between two pieces of woven gauze.

2.4.2. Semilunar vestibular incision technique

After administration of local anesthesia a semilunar shaped incision is placed in the vestibule extending from distal aspect of the lateral incisor to the mesial aspect of the molar. An intracrevicular incision was made following the curvature of the receded gingival margin involving the interdental papilla. A full thickness dissection was performed apically from the intrasulcular incision, and this dissection was connected to the vestibular incision. Coronal mobilization of the flap was considered adequate when the marginal portion of the flap was able to passively reach a level coronal to the CEJ of the tooth. Both L-PRF and A-PRF membranes are placed into the flap and finger pressure is applied over the flap to compress the membranes. Sutures were placed by pulling the flap coronally and periodontal dressing was given. Fig. 2 (A–F).
Fig. 2  Semilunar vestibular incision technique A: Semilunar incision, B: Intrasulcular incision, C: Full thickness mucoperiosteal flap elevation, D: Placement of PRF membranes, E: Interrupted sutures placed, F: Periodontal dressing given.

Fig. 3  Pouch and tunnel technique, A: Intrasulcular incision, B: Tunnel access preparation, C: Intact interdental papilla, D: Placement of A-PRF membrane, E: Placement of L-PRF membrane, F: Periodontal dressing given.

Fig. 4  A: Pouch and tunnel technique pre-operative view, B: Pouch and tunnel technique 6 months post-operative view, C: Semilunar vestibular incision technique pre-operative view, D: Semilunar vestibular incision technique 6 months post-operative view.
2.4.3. Pouch and tunnel technique

After administration of local anesthesia, sulcular incisions were given through each recession area. Care was taken not to extend the incisions till the tip of the interdental papilla. A full thickness mucoperiosteal flap was reflected, extending beyond the mucogingival junction. This was done so as to reduce the tension on the flap to facilitate coronal displacement. Each pedicle adjacent to the recession was undermined gently, without detaching it completely to prepare a tunnel. The undermining of tissues is done to prepare the tunnel by extending it laterally about 3-5 mm. Both A-PRF and L-PRF membranes were placed into the tunnel from the sulcus and finger pressure is applied to compress the membranes and also to mobilize the flap coronally and periodontal dressing is given Fig. 3 (A–F).

2.5. Post-operative instructions

Regular post-operative protocol was followed after the surgical procedure. Patients were recalled for suture removal after 2 weeks. The patients were evaluated periodically for a period of 6 months and all the clinical parameters were recorded.

2.6. Statistical analysis

The data was subjected to statistical analysis using IBM SPSS Software Version 20. Intra group comparison was done by paired T-test and inter group comparison was done by unpaired T-test. Mean, standard deviation and test of significance was calculated for both the groups at baseline, 3 months and 6 months.

3. Results

All the sites healed uneventfully with no post-operative complications. Plaque index and Gingival index decreased significantly in both the groups at baseline, 3 months and 6 months. In both Group A and Group B, there was statistically significant reduction in recession depth, recession width and clinical attachment loss levels at baseline, 3 months and 6 months after the surgery. There was statistically significant increase in the width of keratinized tissue from baseline to 6 months. During inter group comparison the reduction of recession depth, recession width and clinical attachment loss levels

| Table 1 | Mean, standard deviation and test of significance for plaque index and gingival index at baseline, 3 months and 6 months. |
|-----------------------|-------------------------------------------------|
| Clinical parameters   | Baseline | 3 months | 6 months | Difference | P value |
|                       | Mean     | SD       | Mean     | SD        | Mean     | SD     |         |
| Plaque index          | 2.05     | 0.370    | 0.925    | 0.166     | 0.375    | 0.183  | 1.675    |
|                       |          |          |          |           | 0.187    |        | 0.000S   |
| Gingival index        | 2        | 0        | 0.8375   | 0.192     | 0.325    | 0.088  | 1.675    |
|                       |          |          |          |           |−0.088    |        | 0.000S   |

Statistical analysis: paired T test. Statistically significant if P < 0.05*, S-Significant.

| Table 2 | Mean, standard deviation and test of significance for semilunar vestibular incision technique (Group A) at baseline, 3 months and 6 months. |
|-----------------------|-------------------------------------------------|
| Clinical parameters   | Baseline | 3 months | 6 months | P value |
|                       | Mean     | SD       | Mean     | SD        |         |
| RD                    | 2.4375   | 0.417    | 0.1875   | 0.259     | 0.5     |
| RW                    | 2.94     | 0.417    | 0.1875   | 0.258     | 1       |
| CAL                   | 3.4375   | 0.417    | 1.1875   | 0.258     | 1.5     |
| KT                    | 1.625    | 0.517    | 4.0625   | 1.083     | 3.5625  |
|                       |          |          |          |           | 1.083    | 0.000S |

RD – recession depth, RW – recession width, CAL – clinical attachment loss, KT – width of keratinized tissue. Statistical analysis: paired T test. Statistically Significant if P < 0.05*, S-Significant.

| Table 3 | Mean, standard deviation and test of significance for pouch and tunnel technique (Group B) at baseline, 3 months and 6 months. |
|-----------------------|-------------------------------------------------|
| Clinical parameters   | Baseline | 3 months | 6 months | P value |
|                       | Mean     | SD       | Mean     | SD        |         |
| RD                    | 2.4375   | 0.417    | 1.1875   | 0.258     | 1.5625  |
| RW                    | 2.9375   | 0.417    | 1.1875   | 0.258     | 2.0625  |
| CAL                   | 3.4375   | 0.417    | 2.1875   | 0.258     | 2.5625  |
| KT                    | 1.4375   | 0.443    | 2.5625   | 0.678     | 2.075   |
|                       |          |          |          |           | 0.572   | 0.000S |

RD – recession depth, RW – recession width, CAL – clinical attachment loss, KT – width of keratinized tissue. Statistical analysis: paired T test. Statistically significant if P < 0.05*, S-Significant.
and gain of width of keratinized tissue levels were more statistically significant in Group A than Group B (Fig. 4A–B). The results were summarized in the Tables 1–4.

4. Discussion

The ultimate goal of periodontal plastic surgical procedures for recession coverage is the complete regeneration of the supporting components of the periodontium, resulting in complete coverage of the denuded root surfaces in an esthetic as well as a functional manner (Sharma and Yadav, 2015).

Full mouth plaque index Silness and Loe, 1964 and Gingival Index Loe and Silness, 1963 were recorded at baseline, 3 months and 6 months after the surgery. The present study showed a significant reduction in the mean PI scores in both the treatment groups after 6 months postoperatively. This may be due to the regular and frequent recall visits in which the patients underwent regular supragingival scaling, which prevented plaque formation and accumulation. These results are in accordance with studies done by Sato et al. (1993) and Cugini et al. (2000). GI scores showed statistically significant reduction in both the treatment groups, which may be due to the elimination of local etiological factors which harbor numerous pathogenic strains and decrease in inflammation post-surgically. This was in accordance with Becker et al. (1988), Boretti et al. (1995) and Cugini et al. (2000).

In semilunar vestibular incision technique, recession depth, recession width and clinical attachment loss decreased and the width of keratinized tissue increased significantly from baseline to 6 months postoperatively, and these results were in accordance with the study done by Pandit et al. (2015), where they have done semilunar vestibular incision technique on two patients who presented with multiple recessions in the maxillary anterior teeth and got a result of about 90–100% root coverage when the patients were on a follow up for 1 year with a significant increase in the vestibular depth, good color blend and an esthetic marginal morphology. Semilunar vestibular technique provides blood supply from the lateral sides of flap and almost 4 mm of the flap is there to ensure proper vascularity of the flap. This technique facilitates coronal positioning and prevents flap retraction, which is extremely difficult in patients with shallow vestibule. It also increases the width of attached gingiva. Thus, semilunar vestibular incision technique is technically simple causing less discomfort to the patient, no tension and better control and stabilization of the coronally advanced flap which ultimately leads to better esthetic appearance (Pandit et al., 2015).

In the present study, pouch and tunnel technique showed statistically significant reduction in recession depth, recession width and clinical attachment loss and increase in the width of keratinized tissue from baseline to 6 months after the surgery, and these results were in accordance with the study done by Zabalegui et al. (1999) where they have treated multiple gingival recessions with tunnel subepithelial connective tissue graft and reported that there was 100% root coverage in 66.7% of the recessions treated, with a mean root surface coverage of 91.6%, 12 months post operatively. Dani et al. (2014) have also performed pouch and tunnel technique in combination with SCTG in a case series and reported 100% root coverage 1 year post operatively in all the patients. The use of the tunnel technique preserves the papillary height between two mucogingival defects and also helps maintain adequate blood supply to the underlying graft. It provides excellent adaptation of the graft to the recipient site and produces highly esthetic results along with an increase in the thickness of keratinized gingiva (Dani et al., 2014).

Due to decrease in centrifugation speed and an increase in centrifugation time there is higher platelet concentrations in the distal part of clot and a difference in distribution pattern for neutrophilic granulocytes (Ghanaati et al., 2014). Recent studies have shown that neutrophilic granulocytes have tissue regeneration properties (Kolaczkowska and Kubes, 2013; Ley and Laudanna, 2007). They have MMP-9 which play a part in the process of revascularization of the tissue defect by being recruited, for example, by VEGF-A (Kolaczkowska and Kubes, 2013; Christoffersson et al., 2012). Thus, the distribution of neutrophilic granulocytes within the A-PRF clot might be the reason for a better functionality of the transplanted monocytes/macrophages and lymphocytes and their deployment to support tissue regeneration (Ghanaati et al., 2014). In the present study a combination of A-PRF and L-PRF has been used to get advantages of both the platelets and neutrophilic granulocytes along with growth factors. It has been reported that both autogenous PRF and connective tissue graft (CTG) are effective treatment methods for gingival recession defects. As a result, PRF technique can be used as an alternative to the CTG, when factors such as the success rate for gingival recession and patient comfort after surgery are considered (Tunali et al., 2015).

During inter group comparison; there was a greater reduction in recession depth, recession width and clinical attachment loss and increase in the width of keratinized tissue from baseline to 6 months in semilunar vestibular incision technique when compared to that of the pouch and tunnel technique.

| Table 4 | Mean, standard deviation and test of significance for semilunar vestibular incision technique vs. pouch and tunnel technique groups after 6 months postoperatively. |
|---|---|---|---|---|
| Clinical parameters | SVT 6 months | PT 6 months | Mean difference | P value |
| | Mean | SD | Mean | SD | |
| RD | 0.5 | 0.378 | 1.5625 | 0.417 | −1.0625 | 0.0000 S |
| RW | 1 | 0.3779 | 2.0625 | 0.417 | −1.0625 | 0.0000 S |
| CAL | 1.5 | 0.377 | 2.5625 | 0.417 | −1.0625 | 0.0000 S |
| KT | 3.5625 | 1.083 | 2.075 | 0.572 | 1.4875 | 0.004 S |

RD – recession depth, RW – recession width, CAL – clinical attachment loss, KT – width of keratinized tissue. Statistical analysis: Unpaired T test. Statistically Significant if P < 0.05*; S-Significant.
This greater decrease might be due to zero tension of the flap in the semilunar vestibular incision technique due to semilunar shaped incision in the vestibule which inturn caused greater stabilization of the coronally advanced flap (Pandit et al., 2015).

The limitations of this study include small sample size and shorter follow up period. The gingival biotype in the present study has not been accessed, which might have altered treatment outcomes.

5. Conclusion

The present study showed better clinical outcomes of all the clinical parameters in both the groups with greater significance in patients treated with semilunar vestibular incision technique when compared to that of pouch and tunnel technique in combination with A-PRF and L-PRF. This is the first study to use A-PRF for the treatment of gingival recessions. Further long term studies with larger sample size are required to determine the efficiency of these techniques.

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Conflict of interest

None.

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