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Randomized Controlled Trial

Comparative evaluation of effect of injectable platelet rich fibrin with collagen membrane to collagen membrane alone for gingival recession coverage

Patra L *et al.* Effect of VISTA on gingival recession coverage

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Abstract

BACKGROUND

Collagen membrane and platelet rich fibrin have emerged as vital biomaterials in the field of periodontal regeneration. Minimally invasive techniques are being preferred by most periodontist as it is patient compliant, with fewer post-surgical complication as compared to conventional surgical techniques. Thus, in this study we have evaluated the effect of injectable platelet rich fibrin (i-PRF) with collagen membrane and collagen membrane alone using vestibular incision subperiosteal tunnel access (VISTA) technique for gingival recession coverage.

AIM

To compare the efficacy of VISTA using collagen membrane with collagen membrane soaked in injectable PRF for gingival recession coverage.

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METHODS

A split mouth randomized controlled clinical trial was designed with 13 subjects having at least 2 teeth indicated for recession coverage were enrolled in this study. The sites were randomly assigned to control group (VISTA using collagen membrane alone) and the test group (VISTA using collagen membrane with i-PRF). The clinical parameters assessed were pocket depth, recession depth (RD), recession width (RW), relative attachment level, keratinised tissue width (KTW), keratinised tissue thickness (KTT), percentage root coverage.

RESULTS

RD showed statistically significant difference between test group at 3 mo (0.5 ± 0.513), 6 mo (0.9 ± 0.641) and control group 3 mo (0.95 ± 0.51) P value 0.008, 6 mo (1.5 ± 0.571) P value 0.04. RW also showed statistically significant difference between test group at 3 mo (1 ± 1.026), 6 mo (1.65 ± 1.04) and control group 3 mo (1.85 ± 0.875) P value 0.008, 6 mo (2.25 ± 0.759) P value 0.001. Results for KTW showed statistically significant results between test group at 1 mo (2.85 ± 0.489), 3 mo (3.5 ± 0.513), 6 mo (3.4 ± 0.598) and control group 1 mo (2.45 ± 0.605) P value 0.04, 3 mo (2.9 ± 0.447) P value 0.004, 6 mo (2.75 ± 0.444) P value 0.003. Results for KTT also showed statistically significant results between test group at 1 mo (2.69 ± 0.233), 3 mo (2.53 ± 0.212), 6 mo (2.46 ± 0.252) and control group 1 mo (2.12 ± 0.193) P value 0.001, 3 mo (2.02 ± 0.18) P value 0.001, 6 mo (1.91 ± 0.166) P value 0.001. The test group showed 91.6%, 81.6% and 67% root coverage at 1 mo, 3 mo and 6 mo, while in the control group showed 82.3%, 66.4%, and 53.95% of root coverage at 1 mo, 3 mo and 6 mo respectively.

CONCLUSION

The use of minimally invasive VISTA technique along with collagen membrane and injectable form of platelet rich fibrin can be successfully used as a treatment method for multiple or isolated gingival recessions of Miller's class-I and class-II defects.

Key Words: Vestibular incision subperiosteal tunnel access; Injectable platelet rich fibrin; Collagen membrane

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Core Tip: that the use of ⁴⁵ minimally invasive vestibular incision subperiosteal tunnel access technique along with collagen membrane acting as scaffold and chemoattractant with added benefit of injectable form of platelet rich fibrin having the capacity of releasing more growth factors and regenerative cells responsible for tissue regeneration ⁶ can be successfully used as a treatment method for multiple or isolated gingival recessions of Miller's class-I and class-II defects.

INTRODUCTION

Gingival recession is a common feature affecting large populations leading to functional and aesthetic problems. While inflammation is the main etiologic factor for gingival recession but other anatomical factors like thin biotype, abnormal tooth position (positioned too far buccally or lingually, direct trauma associated with malocclusion, aberrant frenal attachment, class-II division 2 malocclusion) and iatrogenic factors like mechanical trauma (impaction of foreign bodies, faulty tooth brushing, poorly designed partial dentures). Subgingival restoration margins, presence of calculus, periodontal disease, smoking also plays role in the aetiology of gingival recession^[1-3].

Gingival recession is being treated using various therapeutic approaches with varying degrees of success which depends on aetiology and treatment approach. Various periodontal surgical techniques for root coverage like free gingival graft (FGG), subepithelial connective tissue graft (SCTG), semilunar flap, coronally advanced flap (CAF), guided tissue regeneration (GTR) are available. Among them CAF with connective tissue graft (CTG) is considered as the gold standard for soft tissue augmentation and periodontal root coverage. It has some disadvantages like harvesting from a donor site, limited tissue availability, increased potential for post-harvesting morbidity^[4].

With the introduction of various minimally invasive tunnelling techniques for gingival augmentation similar results could be obtained. It tries to preserve the interdental papillae, unhampered blood supply and faster wound healing. However, these procedures are quite technique sensitive and may cause tissue trauma to sulcular epithelium leading to unfavourable healing outcomes^[7].

So, to avoid these complications, a new minimally invasive approach for treating multiple gingival recession defects within the maxillary and mandibular aesthetic zone was introduced by Zadeh *et al*^[4] called as vestibular incision subperiosteal tunnel access (VISTA) technique. Complete root coverage was observed for all VISTA treated sites along with 1-2 mm gain in keratinised gingiva at the end of 12th month follow-up period. These improvements were sustained at the 20th month observation period^[4].

Mansouri *et al*^[5] compared the VISTA technique with gold standard coronally advanced flap (CAF) technique using CTG for the treatment of gingival recession defects which showed higher frequency of root coverage in VISTA technique as compared to CAF with CTG. Mohamed *et al*^[6] compared the efficacy of VISTA technique with tunnel technique (TUN) using acellular dermal matrix (ADM) allograft for gingival recession coverage. The 6-mo follow up results showed statistically significant difference in favour of VISTA + ADM technique than TUN + ADM technique. This minimally invasive procedure promises adequate blood supply to the surgical site as it requires a small opening leading to the undermining of periosteum completely free from the area of root coverage, which further enhances in the coronal positioning of the flap passively onto the exposed root surface^[3].

Along with various techniques for root coverage procedure, several grafts such as CTG, ADM allograft, Amniotic membrane, bioactive glass can be advocated for root coverage^[3]. Adjunctive agents like recombinant human growth factor, PRP, PRF have been used to accelerate healing and enhance clinical outcomes^[3,8].

Collagen membrane is one of the materials used for gingival recession coverage which is semipermeable, allowing nutrient passage and gas exchange and it supports cell proliferation *via* its lattice-like structure and cell binding ability. It increases tissue volume as it is naturally absorbed and replaced by host tissue and the chemotactic function encourages host cell migration and attachment thus facilitating primary wound closure and reducing the likelihood of membrane exposure or potential wound/membrane contamination^[9].

Another agent that is commonly used for recession coverage is PRF which is a leukocyte and platelet-rich fibrin biomaterial with a specific composition and 3D architecture that plays an important role in the release of growth factor, immune regulation, anti-infectious activities, matrix remodelling during wound healing, and further serve as a scaffold for tissue regeneration by bearing the feature of acting as a barrier membrane in guided bone regeneration (GBR) and guided tissue regeneration (GTR) procedures^[10-14]. PRF has been utilized for the treatment of extraction sockets,

gingival recessions, palatal wound closure, regeneration of periodontal defects, and hyperplastic gingival tissues^[15].

Initially, PRF formulations were lacking a liquid concentrate of proteins as standardised PRF had the majority of its growth factor encapsulated within its fibrin matrix. Recent advances in the field aim at developing a liquid formulation of PRF with no anticoagulants or fibrin matrix which lead to the development of an injectable formulation of PRF termed injectable PRF (i-PRF) which is a platelet concentrate in a liquid formulation that can be either utilized alone or combined easily with various biomaterials. It has a higher presence of regenerative cells with higher concentrations of growth factors and higher fibroblast migration and also the expression of platelet-derived growth factor (PDGF), transforming growth factor (TGF- β), and type-1 collagen when compared to other formulations of PRF^[16,17].

So, the purpose of the study was to compare the efficacy of the VISTA technique incorporating collagen membrane alone with the VISTA technique with collagen membrane soaked in injectable platelet rich fibrin for gingiva recession coverage.

MATERIALS AND METHODS

The study was recommended by the Institutional Ethics Committee, under IEC/SCBDCH/049/20189 dated September 17, 2019 before its commencement and was conducted in accordance with the declaration of Helsinki of 1975, as revised in 2000. This was an interventional, parallel design, double blinded, randomised controlled trial performed from March 2020 to March 2021 in our department. A written informed consent was obtained from all participants after they received a written and oral explanation of study objectives, risks and benefits. The study was prospectively registered with clinical trials registry (CTRI/2020/06/026141).

Patient selection

Sample size was calculated using G power 3.1.9.2 software (SPSS software India by Norman H Nie in 2015 G Power 3.1.9.2) considering 80% power, 95%CI level with an

effect size of 0.55¹ with a mean probing depth 2.27, 2.08 mm before and after treatment with a standard deviation of 0.34 mm.

Inclusion criteria: (1) Both males and females subject of age ≥ 18 years with dentinal hypersensitivity or impaired aesthetics or difficulty in oral hygiene maintenance associated with gingival recession; (2) Subjects having Miller Class I or II bilateral buccal gingival recession defects measuring ≥ 2 mm on the anterior teeth or premolars, on either arch; (3) Subjects who are not on any medication known to interfere with periodontal tissue health or healing within 6 mo of the study; and (4) Subjects having identifiable cementoenamel junction (CEJ) at recession sites.

Exclusion criteria: (1) History of systemic diseases (*i.e.*, diabetes, autoimmune dysfunction, prolonged cortisone therapy, or chemotherapy) that would contraindicate periodontal surgical treatment; (2) Patients with deleterious habits like the use of tobacco chewing or smoking; (3) History of previous periodontal surgical treatment of the involved sites; (4) Presence of malocclusion and pathologic movement of teeth in involved sites; and (5) Presence of active carious lesions, restorations, or crowns at the CEJ as well as non-vital teeth with radicular grooves and irregularities.

Randomisation and allocation

A simple random sampling technique by coin toss was done by an author (SCR) unaware of the clinical parameters, to decide which side/arch to act as test site and which as to control site of each patient. In sites included in the test group recession sites were placed with collagen membrane soaked with injectable platelet rich fibrin and in the control site only collagen membrane was placed.

Preoperative protocol

After enrolment, all the participants underwent an initial non-surgical therapy including full mouth supra and subgingival scaling and root planning using ultrasonic scalers and hand instruments to ensure a healthy periodontium before the onset of surgical phase. Each of them was then given a standardized set of oral hygiene

instructions both verbally as well as in written format. Alginate impressions were taken 4 wk after signal recognition particle and study casts were poured. An acrylic template was fabricated on the study cast extending one tooth mesial and distal to the tooth indicated for extraction. This template was used as reference for the vertical measurements during the course of study.

Clinical parameters

Clinical parameters were recorded at baseline (immediately before surgery) (Figure 1A and Figure 2A) as well as 1 mo, 3 mo and 6 mo follow up for control and test site groups. The clinical parameters recorded were as follows:

²⁹ Plaque index (PI) Silness P and Loe H (1964).

Gingival index (GI) Loe H and Silness P (1963).

²⁵ Probing depth (PD) measured with a UNC-15 periodontal probe as the distance from the gingival margin to the bottom of the pocket.

Recession depth (RD) ³ was measured as the distance from the cemento-enamel junction (CEJ) to the gingival margin at the mid-buccal surface using the UNC-15 probe.

Recession width (RW) was measured with a UNC-15 periodontal probe oriented horizontally and located at the most apical convexity of the CEJ & horizontal distance between the mesial & distal gingival margin.

³³ Relative attachment level (RAL) was measured mid-buccally with the reference point located at the apical end of the groove in the stent ⁷ to the bottom of the periodontal pocket.

Keratinized tissue width (KTW) was measured from the most coronal extension of gingival margin to the mucogingival line.

Thickness of keratinized tissue was measured by using an endodontic K-file (number-20, colour code- yellow) ⁷ with a silicon stop, perpendicular to the tissue surface 2 mm apical to the gingival margin. After reaching the hard surface, the silicon stop was slid and placed in contact with the soft tissue. ⁷ After removing the file, the distance between

the tip of the file and the silicon stop was measured with a digital calliper accurate to the nearest 0.1 mm.

Percentage of root coverage was calculated according to the formula:

Surgical protocol

After extraoral scrubbing⁹ with 5% povidone-iodine solution, the patient was asked to rinse with 10 mL of 0.2% chlorhexidine digluconate solution for 1 min. Root debridement was done with ultrasonic instrument followed by odontoplasty was carried out where necessary using a rotary finishing bur.¹⁰ The surgical site was anesthetized by local infiltration (2% lidocaine HCL with adrenaline 1:100000). The roots are then conditioned for 2 min with 24% buffered ethylenediaminetetraacetic acid gel to eliminate the smear layer.

Test site-Vestibular incision subperiosteal tunnel access approach began with a¹⁰ vestibular access incision at an optimal position to gain access to the recession defects.¹ The location of the access incision depends on the sites being treated e.g., in cases where both premolars are indicated for recession coverage, the vertical access incision was given in between both the premolars.¹⁰ The incision was made through the periosteum using a No. 15 surgical blade (Bard-Parker) exposing the facial osseous plate (Figure 1B). A special set of patented periosteal elevators (VISTA 1-4)¹⁰ was used to elevate the periosteum and create the subperiosteal tunnel.¹³ The attached gingiva adjacent to the incision was elevated using VISTA 1, and the area that are distant from the incision are elevated with VISTA 2, and interproximal areas were elevated with VISTA 3 and 4 instruments.¹³ With VISTA 2 elevator, the tunnel was extended to at least one tooth beyond the teeth requiring root coverage, also beyond the mucogingival junction, and into the gingival sulcus of the teeth in the involved area, to aid in the mobilization of the mucoperiosteal flap (Figure 1C and D).

The mucogingival complex was coronally positioned using an anchored horizontal mattress suture. Anchored horizontal mattress suture placed at a distance of 2-3 mm from gingival margin using 5-0 black braided suture with 3/8 reverse cutting needle.

These anchored sutures were coronally positioned. The knot of the anchored sutures was moved on the facial enamel surfaces of the involved teeth to check the final position of the coronally advanced mucogingival complex. After that the facial enamel surfaces of each tooth were briefly etched for 15 s, irrigated for 15 s, and dried with air. Thereafter bonding agent was applied over the prepared enamel surface and light cured. Then knots of anchored sutures were secured to the prepared facial aspect of each tooth by placing a small amount of flowable composite resin over the knot and was light cured (Figure 1E). This procedure effectively prevents apical relapse of the gingival margin during the initial stages of healing.

For the i-PRF preparation, first tourniquet was tied around the arm of the patient, the skin over the antecubital vein was disinfected with Surgical spirit. Two tubes of 10 mL whole blood were collected by venipuncture of the antecubital vein. The collected blood was centrifuged at 700 rpm for 3 min ($60\times g$) (according to Miron RJ) at room temperature without any additives in restriction enzyme-mediated integration laboratory centrifuge machine. i-PRF formed at the top layer which was immediately collected into a 2 mL syringe with a 25-gauge needle. Then commercially available collagen membrane (HEALIGUIDE Bio resorbable membrane, Advanced Biotech Products, INDIA) was trimmed according to the size of recession in the experimental site, then the trimmed collagen membrane was soaked with i-PRF for 10 min in a steel bowl and inserted into the experimental site with the help of tissue forceps (Figure 1F). Along with this i-PRF was also injected at the mesial and distal aspects into the periodontal ligament and the facial aspect of the gingiva (Figure 1G). Finally, the vertical access incision was approximated and sutured with 5-0 black braided silk sutures achieving primary wound closure (Figure 1H).

Control site-Similar surgical technique was used to prepare the tunnel on the control site (Figure 2B-E). After that collagen membrane was trimmed according to the size of the recession at the control site and soaked with normal saline for 10 min, then inserted into the tunnel. Similar to test site 5-0 black braided silk sutures was used to close the vertical access incision for achieving primary closure (Figure 2F and G).

Post-operative care

All the patients were prescribed antibiotics and analgesics. Post-operative instructions were given to all patients and kept on a strict oral hygiene maintenance program. The vertical incision suture was removed after 1 wk and anchored sutures were removed after 3 wk post-surgery, respectively. The residual composite resin was removed using 16-flute tungsten carbide burs.

The follow-up was done every month for all the patients. During follow-up oral prophylaxis was done and oral hygiene instructions were reinstituted. The measurements of clinical parameters were taken at 1, 3, and 6 mo postoperatively (Figure 1I and J, Figure 2H and I).

Statistical analysis

The data was analyzed using SPSS Ver 22 for windows, (IBM Corp, Armonik, United States). Descriptive statistics were expressed as mean with standard deviations and proportions. Normally distributed data were analyzed using paired *t*-test for intragroup comparison and unpaired *t*-test for intergroup comparison. Skewed data were analyzed using the Wilcoxon Signed Rank test for intragroup and Mann Whitney U test for intergroup comparison. The level of significance was set at $P < 0.05$.

RESULTS

The study consists of 13 subjects (7 males, 6 females) with the mean age of 36.7 ± 12.44 years (Table 1). All recession sites were divided into two groups, Group-I: Test sites (20 sites in which i-PRF with collagen membrane was used for recession coverage) and Group-II: Control sites (20 sites in which collagen membrane alone was used for recession coverage) (consort flow diagram Figure 3). Sample size was calculated using G power 3.1.9.2 software (SPSS software India by Norman H Nie in 2015 G Power 3.1.9.2).

Mean plaque index scores of test group were 0.625 ± 0.151 , 0.865 ± 0.134 , 0.6 ± 0.133 and 0.54 ± 0.127 and of control group were 0.625 ± 0.154 , 0.835 ± 0.172 , 0.545 ± 0.139 and 0.56 ± 0.134 at baseline, post-operative 1 mo, 3 mo and 6 mo respectively. The plaque scores are statistically not significant at different time intervals in the intergroup comparison (Table 2, Figure 4A). While in the intragroup comparison there was a statistically significant difference between mean scores between each time interval for individual groups ($P < 0.01$). However, there was no statistically significant difference found between baseline and postoperative 3 mo for the test group ($P = 0.204$) and between postoperative 3 mo and 6 mo for control group ($P = 0.379$) respectively (Table 3).

Mean gingival index scores of test group were 0.625 ± 0.164 , 0.89 ± 0.141 , 0.545 ± 0.119 and 0.51 ± 0.149 and of control group were 0.625 ± 0.65 , 0.89 ± 0.18 , 0.575 ± 0.155 and 0.51 ± 0.137 at baseline, post-operative 1 mo, 3 mo and 6 mo respectively. Intergroup comparison of gingival index scores revealed no statistically significant difference between mean scores at different time intervals (Table 4 and Figure 4B). However, there was a statistically significant increase in the gingival index scores between baseline and postoperative 1 mo for both the groups ($P < 0.01$). There was a decrease in gingival index scores at subsequent time intervals for both the groups except between postoperative 3 mo and 6 mo ($P = 0.137$) (Table 5).

Mean probing depth scores of the test group were 1.75 ± 0.444 mm, 2.65 ± 0.489 mm, 2.05 ± 0.489 mm and 1.75 ± 0.444 mm and of the control group were 2.05 ± 0.6 mm, 2.8 ± 0.83 mm, 2.1 ± 0.3 mm and 1.95 ± 0.223 mm at baseline, postoperative 1 mo, 3 mo and 6 mo respectively. In the intergroup comparison between test group and control group, there was no statistically significant difference between mean scores at the different time interval between two groups (Table 6 and Figure 4C). Though there was significant increase in probing depth between baseline and 1 mo and subsequent decrease in probing depth at postoperative 1 mo, 3 mo and 6 mo respectively for test group. Similarly, in the control group, there was an increase in probing depth between baseline and 1 mo. Though there was a decrease in probing depth between postoperative 1 mo

and 3 mo, it was not statistically significant and a significant decrease between postoperative 3 mo and 6 mo. There was no significant difference between baseline & postoperative 6 mo, postoperative 1 mo & 6 mo respectively (Table 7).

Mean recession depth scores of test group were 2.7 ± 0.86 mm, 0.25 ± 0.4 mm, 0.5 ± 0.5 mm and 0.9 ± 0.64 mm and of control group were 2.9 ± 0.71 mm, 0.5 ± 0.51 mm, 0.95 ± 0.51 mm and 1.3 ± 0.57 mm at baseline, 1 mo, 3 mo and 6 mo respectively. In the intergroup analysis, there was no statistically significant difference between mean scores at baseline and 1 mo. However, there was a statistically significant difference in mean recession depth at 3 mo ($P < 0.01$) and 6 mo ($P < 0.05$) between both test and control group (Table 8 and Figure 4D). Within the group analysis, there was statistically significant decrease in mean recession depth in both the groups between baseline and 1 mo ($P = 0.001$); 1 mo and 3 mo ($P = 0.021$; $P = 0.001$) and 3 mo and 6 mo ($P = 0.002$; $P = 0.005$) respectively (Table 9).

Mean recession width scores of the test group were 3.5 ± 0.6 mm, 0.5 ± 0.8 mm, 1 ± 1.02 mm and 1.65 ± 1.03 mm and for the control group were 3.7 ± 0.73 mm, 1 ± 1.02 mm, 1.85 ± 0.85 mm and 2.55 ± 0.75 mm at baseline, 1 mo, 3 mo and 6 mo respectively. In the intergroup analysis there was statistically significant difference between the two groups at 3 mo ($P < 0.01$) and 6 mo ($P < 0.01$) respectively. There was no statistically significant difference at baseline and 1 mo (Table 10 and Figure 4E). In intragroup analysis there was a statistically significant decrease in recession width between baseline and 1 mo ($P = 0.001$) and an increase in the width of recession between 1 mo and 3 mo ($P = 0.025$; $P = 0.004$) in both the groups respectively. Similarly, there was an increase in the recession width between 3 mo and 6 mo ($P = 0.009$; $P = 0.001$) (Table 11).

Mean relative attachment scores for the test group were 7.3 ± 0.8 mm, 5.6 ± 1.3 mm, 5.25 ± 1.29 mm and 5.55 ± 1.09 mm and for the control group was 7.05 ± 0.82 mm, 5.4 ± 0.94 mm, 5.05 ± 0.94 mm and 5.45 ± 0.82 mm at baseline, 1 mo, 3 mo and 6 mo respectively. In intergroup analysis, there was a statistically significant difference between the two groups at 3 mo ($P < 0.01$) and 6 mo ($P < 0.01$) respectively (Table 12 and Figure 4F). There was no statistically significant difference at baseline and 1 mo.

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There was a statistically significant decrease in the attachment level in both the groups between baseline and 1 mo ($P = 0.001$). There was a further decrease in the test group between 1 mo and 3 mo ($P = 0.021$) and between 3 mo and 6 mo for the control group ($P = 0.021$) (Table 13).

Mean width of keratinised tissue scores for the test group were 1.6 ± 0.5 mm, 2.85 ± 0.48 mm, 3.5 ± 0.51 mm and 3.4 ± 0.59 mm and for the control group were 1.35 ± 0.48 mm, 2.45 ± 0.6 mm, 2.9 ± 0.44 mm and 2.75 ± 0.44 mm at baseline, 1 mo, 3 mo and 6 mo respectively. Intergroup comparison, revealed statistically insignificant difference between the two groups at baseline but there was a statistically significant difference at 1 mo, 3 mo and 6 mo respectively (Table 14 and Figure 4G). In intragroup comparison there was a statistically significant increase in the width of keratinized tissue in both the groups ($P = 0.001$) between baseline and 1 mo and between 1 mo and 3 mo respectively ($P = 0.001$; $P = 0.013$) (Table 15).

Mean thickness of keratinised tissue observed for the test group were 1.64 ± 0.237 mm, 2.68 ± 0.233 mm, 2.52 ± 0.211 mm and 2.45 ± 0.252 mm and the control group were 1.61 ± 0.201 mm, 2.11 ± 0.193 mm, 2.01 ± 0.179 mm and 1.91 ± 0.166 mm at baseline, 1 mo, 3 mo and 6 mo respectively. In intergroup analysis there was no statistically significant difference found between the mean thickness of keratinized tissue between the two groups at baseline ($P > 0.05$). However, there was a statistically significant difference at 1 mo, 3 mo, and 6 mo respectively (Table 16 and Figure 4H). In intragroup analysis there was a statistically significant increase in the thickness of keratinized tissue in both the groups at all time intervals ($P = 0.001$) (Table 17).

In the analysis of the percentage of root coverage for the test sites in which I-PRF with collagen membrane was used for recession coverage it was found that at 1st month, it was about 75% of sites had 100% root coverage, 20% had of sites > 50% root coverage and only 5% of sites had 50% root coverage. In 3rd month, 50% of sites had 100% root coverage, 30% had > 50% root coverage and 20% of sites had 50% root coverage. In the 6th month, only 25% of sites remained at 100% root coverage, 25% of sites had > 50% root coverage, 45% of sites had 50% root coverage and 5% of sites had < 50% root

coverage (Figure 5A). While in the analysis of the percentage of root coverage for the control sites in which only collagen membrane was used for recession coverage it was found that at 1st month about 50% of sites had 100% root coverage, 40% of sites had > 50% root coverage and 10% of sites had 50% root coverage. In the 3rd month, only 15% of sites had 100% root coverage, 55% had > 50% root coverage, 20% of sites had 50% root coverage and 10% of sites had < 50% of root coverage. In the 6th month, only 5% of sites remained at 100% root coverage, 30% of sites had > 50% root coverage, 40% of sites had 50% of root coverage and 25% of sites had < 50% root coverage (Figure 5B).

In the overall percentage of root coverage, it was found that in the test group 91.6%, 81.6% and 67% root coverage was found at 1 mo, 3 mo and 6 mo, while in the control group it was found 82.3%, 66.4%, and 53.95% of root coverage at 1 mo, 3 mo and 6 mo respectively (Table 18 and Figure 5C).

DISCUSSION

Gingival recession defects present clinicians with significant therapeutic challenges, including restoration of protective anatomy of the mucogingival complex, reestablishment of the aesthetic balance between soft tissues and adjacent tooth structures and ideally regeneration of the lost cementum, periodontal ligament and supporting alveolar bone^[16]. Although a wide range of therapeutic alternative exist for treatment of isolated or multiple gingival recessions, but according to the available systematic reviews, coronally advanced flap with subepithelial connective tissue graft is the most predictable approach and is considered as the gold standard for root coverage procedures^[19-22].

The large avascular area which usually leads to difficulty in restoring blood supply to the grafted tissue and which is vital for healing, the need for large amount of donor tissue and the presence of non-carious cervical lesions are often associated with multiple gingival recessions compound the problem^[3]. Also, muscle pull during healing often leads to incomplete root coverage or relapse of the recession^[4]. Taking all these factors into consideration, a technique which is minimally invasive, which did not

compromise the blood supply and yet resulted in improvement of all the clinical parameters, the VISTA technique can be an accepted approach^[3,4].

The advantage of VISTA technique over other tunneling approaches and classical techniques of gingival augmentation is the degree of coronal advancement of the gingival margin advocated during the procedure^[3]. Placement of the initial vertical access incision and the subperiosteal tunnel entrance, far from the gingival margin reduces the risk of trauma to the gingiva while at the same time maintains the integrity of the interdental papilla by avoiding papillary reflection and marginal tissue loss of the teeth being treated^[3,5,23]. It also provides a wider access to the surgical region, improves the visualisation through the single incision with no visible scarring, maximising the esthetic outcome^[3,6]. The positioning of gingival margin to the most coronal level of the adjacent interproximal papilla rather than to the cemento-enamel junction with the help of coronally anchored suturing technique on the facial surface of each tooth effectively minimises micromotion of the regenerative site and prevents apical relapse of the gingival margin during the initial stages of healing but compensating for some degree of apical migration during the healing period^[3].

Dandu *et al*^[24] conducted a split mouth randomised controlled trial in 15 patients having bilateral Miller class I and II recession defects. Results revealed mean percentage root coverage of 87.37% + 17.78% with statistically significant gain in width of keratinised gingiva and clinical attachment level obtained at 9 mo. Reddy *et al*^[8] conducted a case series study to evaluate clinical efficacy of VISTA technique in combination with PRF and CTG in the treatment of gingival recession defects. Results obtained showed complete root coverage in all the cases at 6 mo respectively and concluded that VISTA technique overcomes the shortcoming of other treatment options and gives better results for multiple gingival recession defects. Garg *et al*^[25] evaluated the efficacy of VISTA with or without PRF membrane in the treatment of multiple Millers class I and class II gingival recession defects. 100% coverage was obtained in class I sites treated with VISTA approach with or without PRF-membrane. Millers class II recession defects showed 100% coverage with 80%-85% of CAL gain at site treated

with VISTA + PRF membrane as compared to sites treated with VISTA technique only displayed 50% coverage. They concluded that VISTA technique alone is a successful approach for treatment of class-I and II multiple recession defects. Moreover, along with PRF-membrane VISTA technique has proven efficiency for treatment of class - III recession defects. Mansouri *et al*^[5] compared the clinical efficacy of VISTA technique with CTG vs CAF with CTG for the treatment of multiple gingival recession defects. Results revealed significant decrease in recession depth, recession width and clinical attachment level and an increase in keratinised tissue width in both the groups. It was concluded that VISTA, as a minimally invasive approach, was able to treat gingival recession defects and reduce their height and width, yielding results similar to those obtained by the use of CAF as the gold standard procedure for root coverage. Mohamed *et al*^[6] compared between Tunnel technique and VISTA technique for the treatment of multiple gingival recessions with ADM. The percentage of root coverage between VISTA + ADM sites and tunnel + ADM sites was statistically significant in favour of the VISTA + ADM. They concluded that ADM allograft can be recommended as an alternative to connective tissue graft but its combination with VISTA technique is found to be more efficient than tunnel + ADM in treatment of Miller class I and II multiple gingival recessions and leading to favourable root coverage.

Guided tissue regeneration is a reliable method for periodontal regeneration and the introduction of resorbable collagen membranes allowed clinicians to achieve a predictable, new connective tissue attachment over the exposed root surface^[26-30]. Collagen membrane acting as a barrier, mechanically prevents the epithelial cell migration during the initial stages of healing, allowing the regeneration of the treated root surface by connective tissue cells, eventually to the development of a new connective tissue attachment. The cross-linked structure slows the degradation rate allowing the membrane to remain in the site for a sufficient period of time which prevents the apical migration of epithelial cells in late stages of healing thus discouraging the formation of long junctional epithelial attachment and favouring development of connective tissue attachment^[31,32].

Since the introduction of PRF^[33], it has been used in various types of periodontal defects with good results. PRF is being autologous, easy to prepare in a short period of time and little biochemical handling is an advantage. It has a matrix of fibrin which has trapped platelets, leukocytes and cytokines. It acts as a source of growth factors which are released slowly over a period of 7 d and play an important role in recession coverage^[34]. One drawback that limits the applications of PRF is that PRF is obtained as a gel form which is not conducive to be injected^[35-37].

i-PRF also has similar properties as PRF and in addition to that it is available in injectable form. It contains all components of PRF, including platelets, white blood cells and all the clotting factor comprising fibrinogen in an uncoagulated form because of which they are readily available^[38]. The major advantages of i-PRF over other platelet concentrates is due to the "slow speed concept" of blood centrifugation, because of which it contains a greater number of regenerative cells with higher concentrations of growth factors and leukocytes^[39,40]. Leukocytes have been known to play an important role in wound healing and tissue regeneration. With the increased number of cells there is possibly increased release of growth factors like PDGF, epidermal growth factor, TGF- β and insulin-like growth factor-1^[41,42].

According to Miron *et al*^[43] when injectable PRF was compared with PRP in terms of cell proliferation, PRP was more effective than injectable PRF. However, injectable PRF demonstrated significantly better results than PRP did, including cell migration and messenger ribonucleic acid expression of TGF- β , platelet-derived growth factor (PDGF), and collagen type 1a2 at both 3- and 7-d intervals. Also, whereas PRP had completely dissolved over a period of 10 d, injectable PRF formed a small clot as a dynamic gel and maintained release of growth factor for over 10 d. Varela *et al*^[44] observed that i-PRF induces higher cell migration and expression of TGF- β , PDGF and type I collagen which stimulates the differentiation of osteoblasts and deposits mineral matrix. İzol *et al*^[45] investigated the outcome of i-PRF on root coverage of free gingival graft surgery. The result showed a positive effect on the coverage of the root surface. Ucak Turer *et al*^[46] investigated the combined effect of SCTG with i-PRF and SCTG alone in a coronally

positioned flap procedure for the treatment of root coverage and observed that the combined effect of SCTG and i-PRF achieved more amount of keratinized tissue width and showed predictable results in reduced gingival recession. Ozsagir *et al*^[47] evaluated the efficacy of injectable platelet-rich fibrin alone and in combination with microneedling on gingival thickness and KTW in patients with thin biotype. They stated that microneedling has a beneficial result on the augmentation of gingival thickness. Al-Maawi *et al*^[48] analysed the combination of an autologous injectable-PRF matrix as a drug delivery system, with five different xenogeneic collagen-based biomaterials (Mucograft®, Bio-Gide®, Mucoderm®, Collprotect® and BEGO®) histologically. It was found that injectable PRF could be used as a drug delivery system to support GTR/GBR and enhance their biomaterial bioactivity. Chai *et al*^[49] conducted a comparative analysis study to compare the cellular regenerative activity of human dental pulp cells (hDPCs) when cultured with either injectable PRF or traditional PRP. The findings from the study suggested that injectable PRF promoted higher regeneration potential of hDPCs when compared with traditional PRP. Furthermore, injectable PRF also helped in reduction of the inflammatory condition created by lipopolysaccharides and maintained a supportive regenerative ability for stimulation of odontoblastic differentiation and reparative dentin in hDPCs. Bennardo *et al*^[50] conducted a split mouth randomised controlled trial to compare the efficacy of i-PRF and triamcinolone acetonide (TA) injective therapies in patients with symptomatic oral lichen planus (OLP). The results obtained with i-PRF are similar to those obtained with TA. It was concluded that although i-PRF injections do not represent a standard treatment option, they have proved to be equally effective in reducing symptoms and dimensions of OLP lesions.

VISTA technique has been applied for gingival recession coverage using different regenerative materials like CTG^[5,51,52], PRF^[52,53], titanium PRF^[53], ADM^[6], GEM 21S^[24], recombinant human platelet derived growth factor^[4], and Collagen membrane^[54] but, there was no study using i-PRF in combination with collagen membrane using VISTA technique for recession coverage.

In this split mouth randomised clinical trial the full mouth plaque and gingival index scores remained low throughout the study period. It was observed that plaque and gingival index were increased in 1 mo, which could be due to coronally advanced suture held in the facial enamel surface for 3 wk postoperatively leading in difficulty to maintain oral hygiene in the operated region. But there is reduction in plaque and gingival index at 3rd and 6th month which is due to better patient compliance and regular oral hygiene instructions given to the patients thereby enabling improved plaque control efficiency.

The change in mean probing depth in i-PRF with collagen membrane group was statistically insignificant between both groups which is in accordance with observation by Geeti *et al*^[55] Similarly, another study done by Mohamed *et al*^[6] where they used acellular dermal matrix (ADM) for recession coverage, showed reduction in probing depth score. The intergroup comparison in the present study was statistically insignificant at each time intervals which is in accordance with the study done by Subbareddy *et al*^[3].

Recession depth in the present study revealed a significant reduction of the test and control groups respectively at the end of 6 mo postoperatively. This is similar with the case series done by Raja Rajeswari *et al*^[56] There was significant result in the intergroup comparison at 3 mo and 6 mo which is in line with the split mouth study done by Subbareddy *et al*^[3] in which VISTA + PRF was compared with VISTA + SCTG.

Reduction in recession width was statistically significant in each postoperative visit in comparison to baseline for both the groups. This is in agreement with the study done by Mansouri *et al*^[5] in which VISTA was compared with coronally advanced flap procedure using connective tissue graft. The present study shows i-PRF with collagen membrane is equally effective in reducing the width of recession when compared to VISTA with CTG.

There was significant gain in attachment gain in attachment level for both i-PRF + CM and CM groups at 6 mo which is in accordance with Mansouri *et al*^[5]. Improvement

in clinical attachment may be because of recession coverage that results from coronal shift of attachment apparatus during coronally advance flap procedures.

In the present study, width of keratinised gingiva in the subjects of both the groups showed significant increase in 1 mo and 3 mo and it was sustained till 6 mo. These results are in accordance with study done by Mohamed *et al*^[6] though the study used VISTA + PRF for recession coverage. Similarly, a study done by Dandu *et al*^[24] showed gain in width of keratinised gingiva in which VISTA with collagen membrane enhanced with GEM 21S was used for recession coverage.

There was significant gain of mean thickness of keratinised gingiva in test and control groups which is similar to the results of study using VISTA with PRF done by Geeti *et al*^[55] and Raja Rajeswari *et al*^[56].

In the overall percentage of root coverage of the present study it was observed that at 1 mo there was 91% and 82% of root coverage which reduced to 67% and 53% of root coverage at 6 mo for test and control group respectively. It was also observed that at the end of 6 mo 25% (No. 5) of sites had complete root coverage for test group while only 5% of sites (No. 1) had complete root coverage for control group. Similarly, in the study by Mansouri *et al*^[5] mean root coverage achieved was 70.69% and 50% of cases having complete root coverage in the VISTA with CTG group. In a study done by Subbareddy *et al*^[3] showed that in the test group involving VISTA with PRF 30.33% of sites obtained complete root coverage whereas the remaining sites constituting 69.67% partial root coverage.

In the overall assessment of the result of the study, it was observed that probing depth, recession depth, recession width and relative attachment level, test sites have shown similar results to control sites. Width of keratinised tissue, thickness of keratinised tissue and percentage of root coverage, in i-PRF with collagen membrane shown better results than sites where only collagen membrane was used for recession coverage. This can be attributed to VISTA technique as it was a minimally invasive surgery which not only reduces the trauma to the operating site but also preserves the

major blood vessels of the flap and blood supply to the area, resulting in better nourishment of the collagen membrane.

The use of i-PRF is not only helpful for enrichment of collagen membrane with various growth factors responsible for tissue regeneration but also injecting it into the mesial and distal aspects into periodontal ligament and into the facial aspects of gingiva is an added benefit for stimulation of wound healing^[57].

This study must be interpreted with consideration of relatively small sample size (13 subjects), shorter study duration (6 mo).

CONCLUSION

¹⁸Based on the results of the study it can be concluded that the use of minimally invasive VISTA technique along with collagen membrane acting as scaffold and chemoattractant with added benefit of injectable form of platelet rich fibrin having the capacity of releasing more growth factors and regenerative cells responsible for tissue regeneration ⁶can be successfully used as a treatment method for multiple or isolated gingival recessions of Miller's class-I and class-II defects.

ARTICLE HIGHLIGHTS

Research background

Gingival recession is being treated using various therapeutic approaches with varying degrees of success which depends on aetiology and treatment approach. Among them, ⁷Coronally advanced flap technique along with connective tissue graft is considered the gold standard for soft tissue augmentation and periodontal root coverage. But it has some disadvantages like harvesting from a ²donor site, limited tissue availability, and increased potential for post-harvesting morbidity. With the introduction of minimally ⁸invasive vestibular incision subperiosteal tunnel access (VISTA) technique similar results could be obtained. It tries to preserve the interdental papillae, unhampered blood supply, maintains the marginal integrity, minimises micromotion of flap and faster wound healing with no visible scarring maximising the esthetic outcome. This

study is an attempt to find the efficacy of VISTA technique using collagen membrane soaked in autologous injectable formulation of platelet rich fibrin termed as injectable platelet rich fibrin (i-PRF) for the treatment of multiple gingival recession coverage.

Research motivation

The main topic is to compare the efficacy of minimally invasive VISTA technique for the treatment of multiple gingival recession coverage using collagen membrane and collagen membrane soaked in i-PRF. Placement of the initial vertical access incision and the subperiosteal tunnel entrance being far from the gingival margin reduces the risk of trauma to the gingiva while at the same time maintaining the integrity of the interdental papilla by avoiding papillary reflection and marginal tissue loss of the teeth being treated. It also provides wider access to the surgical region, and improves the visualisation through the single incision with no visible scarring, maximising the esthetic outcome. The positioning of the gingival margin to the most coronal level of the adjacent interproximal papilla rather than to the cemento-enamel junction with the help of the coronally anchored suturing technique on the facial surface of each tooth effectively minimises micromotion of the regenerative site and prevents apical relapse of the gingival margin during the initial stages of healing. The use of i-PRF also has similar properties as PRF and in addition to that, it is available in injectable form. It contains all components of PRF, including platelets, white blood cells and all the clotting factors comprising fibrinogen in an uncoagulated form because of which they are readily available. The major advantage of i-PRF over other platelet concentrates is that it contains a greater number of regenerative cells with higher concentrations of growth factors and leukocytes. With the increased number of cells, there is possibly an increased release of growth factors like platelet-derived growth factor, epidermal growth factor, transforming growth factor and insulin-like growth factor-1.

Research objectives

The main objective is to compare the efficacy of the VISTA technique incorporating collagen membrane alone with the VISTA technique with collagen membrane soaked in injectable platelet rich fibrin for gingival recession coverage in terms of clinical parameters like pocket depth, recession width, recession depth, width of keratinised gingiva, thickness of keratinised tissue and percentage of root coverage. The overall assessment of the result of the study, it was observed that probing depth, recession depth, recession width and relative attachment level, test sites have shown similar results to control sites. Width of keratinised tissue, thickness of keratinised tissue and percentage of root coverage, in i-PRF with collagen membrane showed better results than sites where only collagen membrane was used for recession coverage. This can be attributed to VISTA technique as it was a minimally invasive surgery which not only reduces the trauma to the operating site but also preserves the major blood vessels of the flap and blood supply to the area, resulting in better nourishment of the collagen membrane. The use of i-PRF is not only helpful for the enrichment of collagen membrane with various growth factors responsible for tissue regeneration but also injecting it into the mesial and distal aspects of periodontal ligament and into the facial aspects of gingiva is an added benefit for stimulation of wound healing.

Research methods

The data was analysed using SPSS Ver 22 for windows, (IBM Corp, Armonik, United States). Descriptive statistics were expressed as mean with standard deviations and proportions. Normally distributed data were analysed using paired *t*-test for intragroup comparison and unpaired *t*-test for intergroup comparison. Skewed data were analysed using the Wilcoxon Signed Rank test for intragroup and Mann Whitney U test for intergroup comparison. The level of significance was set at $P < 0.05$.

Research results

The result of the study observed that probing depth, recession depth, recession width and relative attachment level, test sites have shown similar results to control sites.

Width of keratinised tissue, thickness of keratinised tissue and percentage of root coverage, in i-PRF with collagen membrane showed better results than sites where only collagen membrane was used for recession coverage. This can be attributed to VISTA technique as it was a minimally invasive surgery which not only reduces the trauma to the operating site but also preserves the major blood vessels of the flap and blood supply to the area, resulting in better nourishment of the collagen membrane. The use of i-PRF is not only helpful for the enrichment of collagen membrane with various growth factors responsible for tissue regeneration but also injecting it into the mesial and distal aspects of periodontal ligament and into the facial aspects of gingiva is an added benefit for stimulation of wound healing.

Research conclusions

VISTA technique has been applied for gingival recession coverage using different regenerative materials like connective tissue graft, PRF, titanium PRF, acellular dermal matrix, GEM 21S, recombinant human platelet derived growth factor, and collagen membrane but, there was no study using i-PRF in combination with collagen membrane using VISTA technique for gingival recession coverage. The results of the study proposed that the use of minimally invasive VISTA technique along with collagen membrane with added benefit of injectable form of platelet rich fibrin having the capacity of releasing more growth factors and regenerative cells responsible for tissue regeneration can be successfully used as a treatment method for multiple or isolated gingival recessions of Miller's class-I and class-II defects.

Research perspectives

This study must be interpreted with consideration of relatively small sample size (13 subjects), shorter study duration (6 mo). long term follow up study with larger sample size is required.

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