Research Protocol

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Dentistry Section

Effectiveness of Titanium Prepared Platelet Rich Fibrin Membrane vs Platelet Rich Fibrin Membrane in the Treatment of Multiple Gingival Recession Defects using M-VISTA Technique: Protocol for a Randomised Clinical Trial

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ABSTRACT

Introduction: The focus of root coverage procedures is now shifting to minimally invasive surgical approaches. Periodontists are being required to adopt newer and more unique procedures to thoroughly cover the exposed roots. Due to the sensitivity of various techniques and the fact that it involves a wide variety of operations and their variants, periodontal plastic surgery provide a substantial challenge. A variety of minimally invasive techniques and platelet concentrates which are abundant in growth factors have been assimilated that provide the added benefit of better and rapid healing.

Aim: This study aims to evaluate the effectiveness of Titanium-Prepared Platelet Rich Fibrin (T-PRF) and Platelet Rich Fibrin (PRF) membrane in Miller's Class-I and Class-II multiple Gingival Recession (GR) using Modified-Vestibular Incision Supra-Periosteal Tunnel Access (M-VISTA) technique.

Materials and Methods: The planned protocol would be randomised clinical trial and will be performed over a period of two years. Twenty-two patients will be selected according to the inclusion criteria, each having multiple GR (>2 mm, Class I or II recession) on buccal/labial aspects of teeth in maxilla or mandible, and would be randomly distributed into the two groups (control and test). The M-VISTA technique would be carried out for root coverage using T-PRF (test group) and PRF (control), respectively as regenerative materials and their effectiveness will be compared by measuring the Pocket Probing Depth (PPD), Clinical Attachment Level (CAL), Relative Gingival Marginal Level (RGML), Recession Depth (RD) and Width of Keratinised Gingiva (WKG). Student's paired and unpaired t-tests will be used to evaluate data from the baseline to three and six months for each group.

Expected Results: The authors expect better results of root coverage using the M-VISTA technique in combination with T-PRF (test group). Better results in terms of PPD, CAL, RGML, RD, and increase in thickness of attached gingiva are expected.

Conclusion: Combined effect of using T-PRF in the M-VISTA procedure is expected to improve the periodontal parameters including, the soft tissue outcomes and the plaque and bleeding index.

Keywords: Biomaterials, Modified vestibular incision supra-periosteal tunnel access, Periodontal plastic surgery, Root coverage

INTRODUCTION

Periodontitis is the world's sixth most prevalent oral disease in humans [1]. Periodontitis can result in the destruction of periodontium leading to the one of the most common clinical findings that is pocket formation and GR [1]. GR is defined as "the displacement of the gingival margin apical to the Cemento-Enamel Junction (CEJ)" [1]. GR can be further classified into localised (restricted to a particular site) and generalised (widespread or multiple sites) GRs [1]. The line of treatment for GR aims to eliminate all causative factors and is then followed by surgical treatment for predictable root coverage [2].

There are various surgical interventions available for GR especially for Miller's Class I and II like the Coronally Advanced Flap (CAF), apically displaced flap, Connective Tissue Graft (CTG), to name a few [1,3]. The main aim for the surgical treatment for GR is to thoroughly cover the exposed roots. Periodontists are being required to adopt newer and more unique procedures with minimal invasive surgical approaches for GR. Due to the sensitivity of its techniques and the fact that it involves a wide variety of operations and their variants, periodontal plastic surgery provides a substantial challenge [4].

The tunneling technique was introduced by Allen AL, which is another key advance to CAF. Following its inception in 1994, numerous procedural changes have been proposed [5]. Zadeh HH presented VISTA in the year 2011, which is a minimally invasive technique for root coverage [6]. Furthermore, the VISTA has been tested in conjunction with a variety of graft materials such as CTG, PRF and PRF membrane with promising results [1,4,5,7].

M-VISTA is a contemporary, minimally invasive approach used for the treatment of both generalised and localised GR defects. The procedure is the modification of the traditional VISTA technique, hence the name. The modification includes the location of incision (instead of a sub-periosteal incision, a supra-periosteal incision is given). This method is specifically precise because it allows more access in the vestibule by giving a single vestibular incision which provides access to an entire region. Thus, it allows a good access and proper visualisation of root and bone morphology and dehiscence. It also improves the wound healing by simplifying the tunneling process [1].

Platelets function as reservoirs for growth-factors and cytokines that are required for soft tissue and bone regeneration, as discovered by Robert Marx in 1971. Choukroun's platelet research resulted in the development of a second-generation platelet concentrate (PRF) in 2001 [8]. The PRF clot forms a strong natural fibrin matrix that has a variety of growth-factors that aid tissue regeneration. The health risks linked with silica containing glass tubes used to collect blood for PRF are still a source of concern. T-PRF was developed to overcome the limitations of PRF [9].

T-PRF was created in 2013 by Tunali using biocompatible titanium tubes [10]. It's a fibrin, rich in platelets and leukocytes that's similar to PRF but has a denser fibrin network than PRF. This denser fibrin structure is essential for extending intra-tissue fibrin resorption and releasing growth-factors in a drop-by-drop fashion over time [11]. PRF's ability to regenerate has been established in various studies [6,8,11]. T-PRF's efficacy profile, on the other hand is scarcely known [10,12].

Hence, this research protocol has been made with a novel approach to use T-PRF as a regenerative material in the M-VISTA technique of root coverage. This randomised clinical trial protocol is made with aim to evaluate the comparative effectiveness of T-PRF membrane and PRF membrane in Miller's Class-I and Class-II multiple GR defects using M-VISTA technique in terms of Root Coverage (RC), gain in CAL, Gingival Thickness (GT) and improvement in the WKG.

MATERIALS AND METHODS

The present study is a randomised clinical trial and will be performed over a period of two years and has started in January 2023. The study is planned to be conducted on 22 participants each having multiple GR (>2 m, Millers Class I or II recession) [8] on buccal/labial aspects of teeth in maxilla or mandible from the Outpatient Department (OPD) of Periodontics and Implantology, Sharad Pawar Dental College, Sawangi (Meghe), Wardha, Maharashtra, India after taking written informed consent. Study protocol has been approved by the Institutional Ethics Committee of the chosen study institute. (Ref.No. DMIMS(DU)/IEC/2022/749). The M-VISTA technique would be carried out for root coverage using T-PRF (test group) and PRF (control), respectively as biomaterials and their effectiveness will be compared.

Sample size calculation: It was done by using the data provided by the previous study of Subbareddy BV et al., by using Statistical Package for Social Sciences (SPSS) version 27.0 open-source calculator [13]. The result of the calculation is 40. Sample size formula for difference between two means:

$N=(za+zb)^2\ (d1^2+d2)/D2$

Where Za is the level of significance at 5% i.e., 95% confidence

interval=1.96, Zß is

power of test= 80%=0.84

d1=SD of CAL in test group=1.47

d2=SD of CAL in control group=0.91

D2=Difference between two means=3.97-2.58=0.76

K=1

n=(1.96+0.84)2 (1.472+0.912/1)//0.762=40 total patients. Hence, 20 subjects would be randomly distributed in each group by computer-generated random numbers.

Inclusion criteria: Multiple Recessions >2 mm (GR depth) on buccal/labial aspects of teeth in maxilla or mandible with Class I or II recession Miller's Classification 1950 [8]. The cases with intraoral radiographic evidence of sufficient interdental bone (a radiolucency of less than 2 mm between the crestal bone and the CEJ) and patients with the presence of gingival biotype with varying thickness of 0.7 to1.5 mm are planned to be included in the study.

Exclusion criteria: Smokers and those who use tobacco products (kharra, gutka,paan, betel nut etc.,), those patients who are unwilling to cooperate, those patients with poor oral hygiene and a plaque score of =1 (Turesky-Gilmore-Glickman Modification of Quigley-Hein Plaque Index 1970) [14]. Patients who report Periodontal surgery treatment history in the study area, lactating mothers or a pregnant lady, those who will show presence of teeth with caries or presence of mobile teeth Grade III and crowding in teeth (at the region of concern) are planned to be excluded from the study.

Study Procedure

Initial therapy: Scaling and Root Planing (SRP), and polishing will be performed on each patient. Wherever necessary, coronoplasty and tooth recontouring will be performed. Patients will be taught how to employ "Modified Stillman's brushing technique" to overcome brushing injury [8]. Oral hygiene instructions such as brushing technique (as mentioned above) will be given to the subjects until their plaque score reaches <1 which will be evaluated again six weeks later. If the patient's dental hygiene isn't maintained, he or she will be excluded from the study. The patient will be educated about the study strategy and given the opportunity to sign a consent form.

Clinical measurements

A) **Indices**

The dental hygiene and gingival health of all patients will be assessed on the day of the surgical treatment, three, six and nine months later.

1. **Turesky-Gilmore-Glickman Modification of Quigley-Hein Plaque Index (PI) 1970) [14]:** Plaque will be examined on the labial/buccal and lingual/palatal surfaces of all teeth after a disclosing agent (Skinner's iodine solution) has been used. This system had the scoring criteria as:

- Score 0: No plaque.
- Score 1: Separate flecks of plaque at the cervical margin of the tooth.
- Score 2: A thin, continuous band of plaque (up to 1 mm) at the cervical margin of the tooth.
- Score 3: A band of plaque wider than 1mm but covering less than one-third of the crown.
- Score 4: Plaque covering atleast one-third but less than two-thirds of the crown.
- Score 5: Plaque covering two-third or more of the crown.

Calculations: The sum of the scores around each tooth will be divided by two to obtain the PI score for the tooth. The PI score per person will be obtained by adding the PI score for all teeth and divided by the number of teeth examined.

2. **Papillary bleeding index by Muehlemann HR 1977 [15]**

On the mesial side, a University of North Carolina (UNC)-15 Probe will be carefully placed into the crevicular sulcus at the base of the interdental papilla and moved coronally to tip of the papilla. On the distal aspect of the same papilla, the procedure will be repeated. On a scale of 0-4, the severity of any resulting bleeding will be recorded.

B) **Clinical parameters**

The 'CAL', 'RGML', 'RD', 'WKG', all of which will be assessed using a UNC-15 probe and GT will be measured. Acrylic stents will be made to integrate the clinical parameters and will be used as a reference point enveloping the occlusal surfaces of the test tooth. The periodontal probe is inserted at an angle to reach the deepest area of the defect. Burs will be used to create longitudinal grooves on the stent, which is going to be used as a guide for the periodontal probe. RD will be calculated from cemento-enamel junction to the margin of gingiva. WKG will be achieved by calculating the sulcular depth plus the attached gingiva by UNC-15 Probe.

Clinical measurements will be recorded in the areas to be treated on the day of the procedure and three, six and nine months postoperatively. The GT will be measured with a No. 15 endodontic spreader and topical anaesthetic, this measurement is taken in the relevant area. The spreader is inserted perpendicular to the gingiva through the gingival edge's apical 1.5 mm and thrust forward till it reaches the bone and then secured using a rubber stop. The distance between the rubber stop and the endochuck tip is measured with a Vernier Calliper.

C) **Radiographic measurement**

Intraoral Periapical (IOPA) and grid specification for evidence of sufficient interdental bone.

Preparation of T-PRF Membrane

Ten millilitre of blood sample will be collected from antecubital vein by venipuncture within one minute from the participants for the preparation of T-PRF. The samples will be immediately transferred within 30 seconds in the Titanium test tubes and placed in the centrifugation machine. It will be centrifuged at 2700 rpm for 12 minutes. Following centrifugation, the T-PRF clot will be obtained. The fibrin clot that has developed in the middle of the tube will be removed, and the red blood cell remnants will be discarded. The clot will be moved to the PRF box and pressed in order to obtain T-PRF membranes [16].

Preparation of PRF Membrane

Using a 24-gauge needle, ten-millimetre blood will be drawn from antecubital vein and the blood samples will be collected in 10 mL glass test tubes and centrifuged for 10 minutes at 3000 rpm on a table centrifuge equipment. The fibrin clot that has developed in the middle of the tube will be removed, and the red blood cell remnants will be discarded. The clot will be moved to the PRF box and pressed in order to obtain PRF membranes [8,17].

Test group surgical procedure: Prior to surgery, patients will be directed to rinse their mouths with a mouthwash containing 0.2% chlorhexidine gluconate for one minute. Exposed root surfaces will be scaled and planned using Gracey curettes after local anaesthetic is administered. Flowable composite will be applied on the mesial side and distal side of the teeth to be treated (without etching, interdentally) to facilitate the sling sutures.

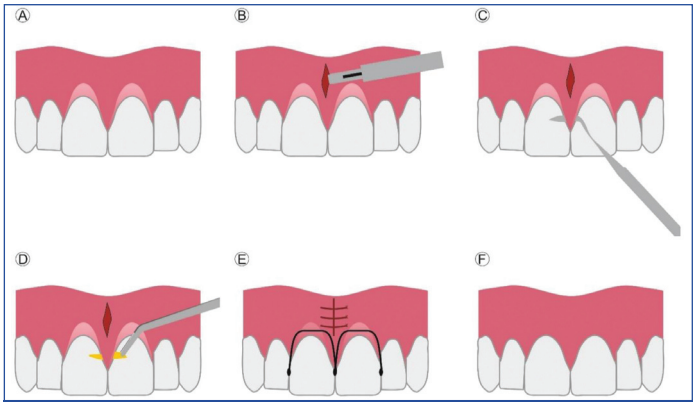
The modified procedure will begin with a long vertical incision made with a no.15 blade starting 1-2 mm from the marginal gingiva and will extend all the way to the periosteum and just past the mucogingival junction and the incision will be created in the most central region, taking into account the extension of the teeth to be treated. Following that, all intracrevicular incisions will be made with a no.15 blade. These incisions will extend to atleast one tooth, beyond the ones to be treated. A full-thickness tunnel will be constructed, extending beyond the mucogingival border into the alveolar mucosa supra-periosteally. This will be accomplished by releasing the tunnel-papillae complex fully through a vertical incision and then gingival borders, allowing for passive coronal replacement. Using 4.0 silk suture. sling sutures will be engaged 2-3 mm apical gingival border of each tooth. After coronal stabilisation, a freshly made T-PRF membrane will be introduced through the tunnel using a small periosteal elevator and uniformly spread across recession defects. The vertical incision will be sutured for the primary closure after the membrane has fully adapted. The Coe-Pak™ will cover the entire surgical site [16,18].

[Table/Fig-1] shows a diagrammatic representation of M-VISTA technique.

Control group surgical procedure: The surgical procedure for the control site will be similar to the procedure for the test site, with the exception that PRF membrane that will be used over the exposed root surfaces.

Postoperative Care

After the treatment, a periodontal pack (Coe pak™) will be placed on the surgical sites. Systemic antibiotic will be given. Subjects will be told not to brush their teeth at the treated site for three weeks after surgery. All patients will be taught to rinse with Chlorhexidine (CHX) gluconate (0.2%) Hexidine (ICPA Health Products ltd.,) twice daily. The periodontal pack will be removed 7 days after the treatment. The sutures will be removed after 14 days and saline irrigation will be done. Before brushing using Modified Stillman's technique, patients will be directed to clean the treated area with cotton dipped in CHX 0.2% for another seven days. The patients will be recalled three, six and nine months after the procedure.



[Table/Fig-1]: A diagrammatic representation of the M-VISTA technique (image created by authors using Coral Draw Software). A) Recession defect; B) Incision made supra-periosteally; C) Preparation of the tunnel; D) Placement of T-PRF/PRF membrane; E) Sutures; F) Root coverage is achieved.

STATISTICAL ANALYSIS

Mean and SD will be calculated for the above-mentioned parameters. Mean data will be analysed using a standard statistical approach for statistical significance. Unpaired and paired t-tests will be used to assess data from the baseline through three, six and nine months for each group. The result is considered not significant if the value of probability is larger than 0.05. It is considered significant if it is less than 0.05. The p-value <0.05 is considered significant.

EXPECTED RESULTS

When the M-VISTA approach is combined with T-PRF, The authors anticipate improved and faster outcomes for root coverage. Better results are anticipated in terms of "PPD," "CAL," "RGML," "RD," "plaque and bleeding indices" and an increase in the thickness of attached gingiva.

DISCUSSION

There are various studies done on tunneling procedures after its inception by Allen AL in 1994 [5], although numerous procedural changes have been proposed [1,6,19-21]. Zadeh HH presented VISTA, which is a minimally invasive technique [6]. A modification of this was then introduced, known as M-VISTA. A case series was conducted by Fernandez-Jimenez A et al., in which the results of the M-VISTA procedure in patients with multiple GR (Miller class III) after six months was evaluated. The result of this study showed mean root coverage of 58.72% after the intervention, with total root coverage in 29% of the GR cases. The authors concluded that M-VISTA may have various advantages over other techniques for class III recession [1].

Mitra DK et al., in their split mouth Randomised Control Trial (RCT) study evaluated the clinical and radiographic effects of autologous T-PRF and PRF in the treatment of infra bony defects, as well as the histologic differences between both the PRF techniques. Clinical parameters such as RAL, PPD were evaluated at baseline, three months and nine months. The result of this study shows reduction in PPD and RAL at three months and nine months. The authors concluded that in an intragroup comparison, clinical metrics and radiographic outcomes with both groups showed considerable improvement. T-PRF displayed denser fibrils in light and scanning electron microscopy than PRF, according to histological analysis [11].

Uzun BC et al., in their study compared the effects of autogenous T-PRF and CTG for the management of multiple GRs. Before surgery as well as during 6- and 12-month follow-up exams,

clinical periodontal indices, Keratinised Tissue Width (KTW), GT, and RD were noted [12]. The visual analogue scale and healing index values were additionally evaluated. The results of this study demonstrated that the mean root coverages were 93.29% and 93.22% in the T-PRF and CTG groups, respectively at 12 month interval. Furthermore, the mean amounts of KTW increased by 1.97 and 0.75 mm in the T-PRF and CTG groups. T-PRF is safe and effective for treatment of multiple Miller Class I/II GR defects. The clinical relevance of this was that T-PRF can serve as a good autogenous alternative to CTG, which is the gold standard for root coverage [12].

The M-VISTA approach was also utilised to repair soft tissue deficit surrounding an implant supported restoration in a case report by Lee CT et al., [20]. A 25-year-old systemically healthy woman, with a tissue deficiency around a single tooth implant in the anterior portion of maxilla was treated using the M-VISTA technique [22]. There was increased tissue height and width around the implant supported restoration in the aesthetic zone. Thus concluding, it can be used to treat soft tissue deficit in the future [20,23].

CONCLUSION(S)

Combined effect of using T-PRF in the M-VISTA procedure will expect novel outcomes with reduced time for regeneration. This study plan will serve many advantages in terms of improving the periodontal parameters including, the soft tissue outcomes at a faster rate.

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