

## Study Protocol

### **Evaluation of effectiveness of Platelet Rich Fibrin Matrix (PRFM) membrane and Platelet Rich Fibrin (PRF) membrane using Vestibular Incision Subperiosteal Tunnel Access (VISTA) Approach technique for the Treatment of Multiple Gingival Recession Defects in Humans – A Study Protocol**

#### **Abstract:**

**Background:** To evaluate the effectiveness of Platelet Rich Fibrin Matrix (PRFM) membrane and Platelet Rich Fibrin (PRF) membrane using Vestibular Incision Subperiosteal Tunnel Access (VISTA) Approach method for the management of multiple gingival recession (GR) defects in humans.

**Methodology:** 20 subjects with gingival recession will be enrolled. 10 patient will be randomly allotted to test group (PRFM membrane) or control group (PRF membrane). Primary outcome will be gain in root coverage (RC) and secondary outcome will be Plaque index (PI), Papillary bleeding index (PBI), probing pocket depth (PPD), relative attachment level (RAL), relative gingival margin level (RGML), recession depth (RD), gingival thickness (GT) and width of keratinized gingiva (WKG). Clinical evaluation will be performed at 3 & 6 months of therapy.

**Expected results:** When all the parameters will be compared at 6 months post-operatively to baseline data, both the treatment group (test and control) will be expected to show significant gain in RC, RAL gain, PPD reduction, decrease in RD, increase in GT and WKG. PRFM membrane will result in significant gain in RC, RAL gain, PPD reduction, and decrease in reduction depth, increase in GT and WKG at 6 months when compared to PRF membrane.

**Conclusion:** PRFM membrane will be effectual in management of gingival recession. PRFM membrane is expected to have significantly greater outcome as compared to PRF membrane group.

**Q1 Keywords:** Vestibular Incision Subperiosteal Tunnel Access, Gingival Recession, Platelet Rich Fibrin Matrix membrane, Platelet Rich Fibrin membrane, Root Coverage

#### **Introduction:**

In routine dental practice, gingival recession (GR) defects are frequently encountered and presents persistent challenges for their management owing to increasing awareness of patient that cause substantial functional and esthetic concern. “GR is defined as the displacement of marginal tissue apical to the cementoenamel junction (CEJ)”.[1] Root coverage procedures are indicated to correct the areas of localized or generalized soft-tissue recession. [2] The treatment of GR is a common request in daily periodontal practice mostly due to esthetic reasons. [3] Recession defects can be isolated or multiple. First line of treatment includes removal of etiological factors followed by surgical correction.[4] Several periodontal plastic surgical procedures have been documented for Miller’s Classes I and II recession defects and negotiate patient’s discomfort due to hypersensitivity, food lodgement, fear of tooth loss,

etc.[5] Evidence echoes that coronally advanced flap (CAF) based techniques are regarded as reliable method for complete root coverage (CRC) while managing isolated GR defect. A primary goal of periodontal therapy is to reduce PPD to limit the risk of local infection. [6] However, there is consistent lack of evidence for coverage of multiple recession defects (MRD).[7]

The surgical treatment for MRD include connective tissue graft,[8] guided tissue regeneration membrane,[9] enamel matrix derivative,[10] acellular dermal matrix allograft,[11] and platelet-rich fibrin (PRF) [7] membrane.

Vestibular Incision Subperiosteal Tunnel Access (VISTA) is a recent, minimally invasive approach used for the management of both multiple and isolated contiguous recession defects.[12] This method is characteristically distinct in that it allows more access in the vestibule and a single vestibular incision can provide access to an entire region. This permits visualization of root and bone morphology or any dehiscence. Additionally, the delicate margin of gingiva has a reduced possibility of trauma, since the line of incision is away from the area of defect, decreasing the invasiveness of the procedure. Treatment with GR defects with VISTA, in conjunction with several bone grafts, leads to gain in tissue thickness along with RC over the exposed root surfaces.

In the field of dentistry a recently developed growth factor delivery system is autologous platelet rich fibrin matrix (PRFM). PRFM has dense concentration of platelets as compared to normal human blood clot. The alpha granules of these growth factors (GF) affect the cells and formation of these tissues involved in the healing of wound. The regeneration of bone and soft tissue has shown an immense potential role in regenerative therapy due to its robust release of GF which are also pivotal components of wound healing process through signaling transduction mechanism.[13] Carroll et al. in 2005 had revealed, in vitro, that the viable platelets in PRFM released six GF mainly epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) in around the same concentration for the seven days duration.[14]

PRF membrane is a fibrin 3-D polymerized matrix in which leukocytes platelet, circulating stem cells, cytokines, and GF are confined and it will help as a resorbable membrane. Hence, it can be used as regenerative therapy in periodontal patients [15]. PRF membrane placement in GR defects can be utilized to establish the function of the properties of the labial gingiva of maxillary and mandibular teeth and re-establishing the integrity & continuity of the zone of KG. Beneficial effects of PRF have been studied in various surgical procedures like sinus floor augmentation during implant placement in multiple gingival recessions with coronally displaced flap and in facial plastic surgical procedures. [16] Jankovic et al[17] conducted a randomized controlled trial and stated that the use of PRF membrane in management of GR provided satisfactory clinical outcomes, and accelerate healing of wound and reduces discomfort of patient in comparison with CTG treated GR defects. Martinez-Zapata et al [18] in their systematic review stated that autologous plasma rich in platelets enhanced GR.

Therefore, the aim of parallel randomized clinical trial is to know if there is root coverage (RC), gains in clinical attachment level (CAL), gingival thickness (GT) & improve width of the keratinized gingiva (WKG) using Platelet rich fibrin matrix (PRFM) membrane in Miller Class I and II multiple gingival recession defect in humans.

### **Objectives:**

1. To evaluate the effectiveness of PRFM membrane using VISTA technique with respect to RC, gains in CAL, GT and improvement in the WKG.
2. To evaluate the effectiveness of (PRF) membrane using VISTA technique in terms of RC, gains in CAL, GT and improvement in the WKG.
3. To compare the effectiveness of PRFM membrane with that of PRF membrane using VISTA technique in terms of RC, gains in CAL, GT and improvement in the WKG.

### **Material and Methods:**

20 subjects with labial or buccal GR defects will be chosen from the outpatient Department of Periodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha using following criteria:

#### **Inclusion Criteria:**

1. Presence of multiple GR (>1) on the labial or buccal surfaces of the teeth having Miller's Class I or II defects either in maxilla or mandible
2. Presence of  $\geq 2$  mm GR depth
3. Radiographic evidence of sufficient interdental bone (the distance between the crestal bone and CEJ as  $\leq 2$ mm).<sup>Q2</sup>
4. Presence of adequate WKG.

#### **Exclusion Criteria:**

1. Patients using tobacco products and smokers.
2. Un-cooperative subjects.
3. Subjects with unsatisfactory oral hygiene after nonsurgical therapy and showing plaque score  $\geq 1$ .
4. History of periodontal surgical treatment in quadrant selected for the study.
5. Pregnant lady or lactating mother.
6. Presence of badly caries teeth
7. Presence of mobile teeth.

### **Study Design:**

Twenty patients, each with minimum 2 adjacent GR defects will be selected for the study. It is a double-blinded study. Prior to surgery selected patients will be randomly assigned to either PRFM membrane group or PRF membrane, each group will be consisting 10 patients respectively.

### **Initial Therapy:**

Each patient will undergo scaling and root planning along with polishing. They will also receive oral hygiene instructions. Coronoplasty and recontouring of teeth will be performed wherever needed. Patient will be demonstrated to use Modified Stillman's brushing technique to reduce the brushing injury. Oral hygiene instructions will be given till the patients attain plaque score of  $\leq 1$ . The patient's plaque control and tissue response will be re-evaluated 6 weeks later. [19] If the patient's oral hygiene is not maintained then the patient will not be included in the study. The information about the purpose of study will be given to patient and informed consent will be signed from patient.

Study protocol will be first accepted by Ethical Committee of Datta Meghe Institute of Medical Sciences, Sawangi(M), Wardha

### **Clinical Measurements**

Clinical records like (PI), (PBI), (PPD), (RAL), (RGML), (RD), WKG and GT will be noted at the day of surgery, three and six months after surgery. Primary outcome will be gain in RC and secondary outcome will be PI, PBI, PPD, RAL, RGML, RD, GT and WKG.

#### **A. Indices:**

Patient's gingival status and oral hygiene will be evaluated at the day of surgical procedure 3 and 6 months.

1. Plaque Index will be measured using Turesky - Gilmore - Glickman Modification of Quigley-Hein 1970 [20]
2. Papillary Bleeding Index will be measured using Muhlemann H.R 1977 [21]

#### **B. Probing Measurements:**

The clinical parameters will be measured for evaluation of the results are the PPD, RAL, [22] RGML, RD, WKG and GT which will be recorded using a UNC-15 probe. To assimilate the clinical parameters, acrylic stents will be made on patient's casts. An acrylic stent covering the occlusal surfaces of test tooth will be used as reference point. To extend periodontal probe to the deepest area of defect, it inserted at an angle. With the help of burs, longitudinal grooves will be made on the stent which will be used as guide for periodontal probe. GT will be calculated 3 mm below the margin of gingiva, under topical anesthesia with the help of endodontic reamer and rubber stopper. RD will be calculated from cement-enamel junction to the margin of gingiva. WKG will be achieved by calculating the sulcular depth plus the attached gingiva by UNC-15 Probe. These clinical measurements will be noted on the treated areas at the day of surgery, 3 and 6 months.

#### **Preparation of PRFM membrane:**

Ten milliliter of blood sample will be collected from antecubital vein by venipuncture within 1 min from the participants for the preparation of PRFM. The samples will be immediately transferred within 30 s in the Meresis PRFM kit (\*R-4C, REMI Laboratory Instruments, Mumbai, India) and placed in the centrifugation machine. It will be centrifuged at rpm of 3000 for 10 min using single-spin centrifugation. Following centrifugation, the PRFM clot will be obtained. [23] The clot will be transferred to PRFM box and compressed to obtain PRF membranes.

#### **Preparation of PRF membrane:**

10mL of blood sample will be withdrawn from antecubital vein with the help of 24-gauge needle. In 10 ml glass test tubes the blood samples will be collected and immediately centrifuged at 2,700 rpm for 12 minutes by using a table centrifuge machine. The fibrin clot formed in the middle part of the tube will be taken, and the remnants of red blood cells will be discarded. The clot will be shifted to the PRF box and pressed so that PRF membranes can be obtained.

#### **Surgical Procedure for Test group:**

Prior to the therapy, the patients will be explained to rinse the mouth with (0.2 %) Chlorhexidine gluconate for 1 minute. The surgical procedure accentuated infection control

and complete asepsis. After induction of 2% local anaesthesia, the exposed root surfaces will be carefully planed<sup>Q3</sup> with ultrasonic instruments followed by curettes. Under local anaesthesia, full thickness 8–10 mm vertical incision will be given in the vestibule mesial or distal to the surgical site treated. This incision will not extended up to gingival margin and served as door for extension of subperiosteal tunnel. This tunnel exposes the osseous plate and extending by Orbans knife up to one or two teeth beyond the recession site undergoing surgical correction. Moreover, the subperiosteal tunnel will be extended into interpapillary region without piercing the papillary tip. Slow apical movement of knife through papillary region commute to the vestibular tunnel and allow the coronal movement of mucogingival complex covering the MRDs. This subperiosteal tunnel will be repositioned coronally and adapted passively to CEJ covering the recession defects. Coronal anchored sutures will be placed engaging the 2–3 mm apical gingival margin of individual tooth using<sup>Q4</sup> 4-0 silk suture. The suture will be secured with the help of composite resin button to prevent apical relapse of the gingival margin, at the midcoronal point of the facial aspect of each tooth. Once coronal stabilization will be achieved, freshly prepared PRFM membrane will be inserted through the tunnel using the small periosteal elevator and spread it uniformly on recession defects. After complete and proper adaptation of the membrane the vertical incision will be sutured for the primary closure. Complete surgical site will be covered with Coe-pack. [24]

#### **Surgical procedure for Control group:**

The surgical method for the control site will be similar to the method of test site except only PRF membrane will be placed over the sites with exposed root surfaces.

#### **Post-operative care:**

After procedure, periodontal pack will be positioned on the recipient site as well as donor site. Non-steriodal anti-inflammatory<sup>Q5</sup> Tab. Ibugesic Plus (Ibuprofen 325 mg + Paracetamol 400 mg), thrice a day & systemic antibiotic Capsule Mox (Amoxicillin 500 mg), thrice a day will be given for 5 days during post-surgical period. Subjects will be explained not to brush the teeth for 3 weeks post-surgery at the treated sites. All subjects will be trained to rinse with (0.2%) chlorhexidine gluconate twice daily, for 14 days.

After 7days of procedure, periodontal pack will be removed. At this time the healing is observed. After 14 days the sutures will be removed. After irrigation with saline, polishing will be done with the help of polishing paste and rubber-cup, without traumatizing the treated site. Patients will be explained to wipe the treated area with cotton dipped in (0.2%) chlorhexidine gluconate for another 7days and later brushing with Charter's method. The subjects will be recalled at one, 3 and 6 months following surgery.

#### **Statistical Analysis:**

The mean and standard deviations (Mean  $\pm$  SD) measures will be recorded. Mean data will be examined by means of a standard statistical method for statistical significance. The data from the baseline to 3 and 6 months per group will be assessed by using Students paired t-test. Assessments among the treatment groups at baseline, three, & six months will be done by means of Student's unpaired t-test. If the probability [p] value is  $> 0.05$ , then the result will be non-significant, and if  $< 0.05$ , it will be considered significant.

#### **Expected Results :**

When all the parameters will be compared at 6 months post-operatively to baseline data, both the treatment group (test and control) will show significant gain in RC, RAL gain, PPD reduction, decrease in reduction depth, increase in GT and WKG. PRFM membrane will

result in significant gain in root coverage, RAL gain, PPD reduction, decrease in recession depth, increase in GT and WKG at 6 months when compared to PRF membrane.

### **Discussion:**

Garg et al (2017) in their case series had evaluated the efficacy of VISTA with or without PRF membrane in management of multiple Classes I and III recession defects. 4 subjects with thirty & forty years of age were included in this study. In all subjects recession defects were treated using the VISTA approach with or without PRF-membrane. Clinical measurements like recession depth (RD), recession width (RW), PPD and CAL were measured at baseline & six months post-op. Class I recession defects treated with VISTA technique alone showed better result. On the other hand, subjects with Class III recession defects who were treated with VISTA + PRF-membrane revealed a better RD reduction and CAL gain in comparison to sites with VISTA only. Therefore the authors concluded that VISTA alone is a suitable procedure for the management of Class I MRDs. For Class III recession defects, inclusion of PRF-membrane gives an enhanced result in terms of RD reduction & CAL gain six months post-op.

Gil et al (2018) in their study observed the relationship among initial site-specific characteristics of subjects with multiple GR & result of RC. Pre and post therapy study models of twenty-one subjects with multiple GR defects, managed with VISTA, were examined. 3-D examination of superimposed before and after surgery images was recorded. Surface and linear RC were recorded. A multistage statistical analysis was done. The mean percentages of linear ( $96.2 \pm 13.1\%$  &  $84.3 \pm 14.4\%$ ), root surface area coverage ( $92.1 \pm 12.0\%$  and  $78.6 \pm 15.7\%$ ) for Miller Class I, II and III recessions were calculated. Initial RW, Initial RD, posterior tooth type and root prominence were found to be negatively interrelated with root surface and linear area coverage while initial gingival margin thickness was found to be positively related with root surface and linear area coverage. The authors concluded the importance of negative and positive area-specific characteristics that may have efficacy in determining the RC.

Do J H (2019) in his study described a minimally invasive surgical approach using the VISTA and the suture called the sub-periosteal sling (SPS) to secure the connective tissue graft (CTG) for periodontal surgery. The SPS suture involves and secure the CTG against the tooth, which reduces the risk of graft mobility. The author concluded that this technique appears to be more suitable and promising for the treatment of localized GR. Other studies on PRF were reviewed[25-27].

### **Conclusion:**

The combination of PRFM membrane will be effective in the treatment of GR. PRFM membrane will have significant clinical outcome as compared to GTR membrane group.

### **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## References:

1. American Academy of Periodontology. Glossary of Periodontal Terms. 3rd ed. Chicago: American Academy of Periodontology; 1992.
2. Nandanwar J, Bhongade ML, Puri S, Dhadse P, Datir M, Kasatwar A. Comparison of effectiveness of hyaluronic acid in combination with polylactic acid/polyglycolic acid membrane and subepithelial connective tissue graft for the treatment of multiple gingival recession defects in human: A clinical study. *Journal of Datta Meghe Institute of Medical Sciences University*. 2018 Jan 1;13(1):48.
3. Jaiswal PG, Puri SS, Bhongade ML. Evaluation of effectiveness of subepithelial connective tissue graft in combination with coronally positioned flap in the treatment of isolated gingival recession in esthetic areas by using surgical microscope. *Journal of Datta Meghe Institute of Medical Sciences University*. 2017 Jan 1;12(1):79.
4. Chan HL, Chun YH, MacEachern M, Oates TW. Does gingival recession require surgical treatment? *Dent Clin North Am* 2015;59:981-96.
5. Chambrone L, Sukekava F, Araújo MG, Pustiglioni FE, Chambrone LA, Lima LA. Root-coverage procedures for the treatment of localized recession-type defects: A Cochrane systematic review. *J Periodontol* 2010;81:452-78.
6. Chavan RS, Bhongade ML, Tiwari IR, Jaiswal P. Open flap debridement in combination with acellular dermal matrix allograft for the prevention of postsurgical gingival recession: A case series. *Int J Periodontics Restorative Dent*. 2013 Mar 1;33(2):217-.
7. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: A 6-month study. *J Periodontol* 2009;80:244-52.
8. Chambrone LA, Chambrone L. Subepithelial connective tissue grafts in the treatment of multiple recession-type defects. *J Periodontol* 2006;77:909-16.
9. Zadeh HH. Minimally invasive treatment of maxillary anterior gingival recession defects by vestibular incision subperiosteal tunnel access and platelet-derived growth factor BB. *Int J Periodontics Restorative Dent* 2011;31:653-60.
10. Aroca S, Keglevich T, Nikolidakis D, Gera I, Nagy K, Azzi R, et al. Treatment of class III multiple gingival recessions: A randomized-clinical trial. *J Clin Periodontol* 2010;37:88-97.
11. Modaresi M, Wang HL. Tunneling procedure for root coverage using acellular dermal matrix: A case series. *Int J Periodontics Restorative Dent* 2009;29:395-403.
12. Zadeh HH. Minimally invasive treatment of maxillary anterior gingival recession defects by vestibular incision subperiosteal tunnel access and platelet-derived growth factor BB. *Int J Periodontics Restorative Dent* 2011;31:653-660.
13. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR, et al. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:638-46.8. Simon.
14. Carroll RJ, Amoczky SP, Graham S, O'Connell SM. Characterization of autologous growth factors in Cascade platelet rich fibrin matrix (PRFM). Edison NJ: Musculoskeletal Transplant Foundation; 2005.
15. Tanya J, Thomas BS (2012) Platelet rich fibrin membrane for recession coverage. *J Dent* 2:223-237
16. Dambhare A, Bhongade ML, Dhadse PV, Sehdev B, Ganji KK, Thakare K, Murakami H, Sugita Y, Maeda H, Alam MK. A randomized controlled clinical study of autologous platelet rich fibrin (PRF) in combination with HA and beta-TCP or HA

- and beta-TCP alone for treatment of furcation defects. *Journal of Hard Tissue Biology*. 2019;28(2):185-90.
17. Jankovic S, Aleksic Z, Klokkevold P, Lekovic V, Dimitrijevic B, Kenney EB, et al. Use of platelet-rich fibrin membrane following treatment of gingival recession: A randomized clinical trial. *Int J Periodontics Restorative Dent* 2012;32:e41-50.
  18. Martínez-Zapata MJ, Martí-Carvajal A, Solà I, Bolibar I, AngelExposito J, Rodnguez L, et al. Efficacy and safety of the use of autologous plasma rich in platelets for tissue regeneration: A systematic review. *Transfusion* 2009;49:44-56.
  19. Sehdev B, Ganji KK, Bhongade ML, Toriya J, Imanishi T, Shoumura M, Alam MK. Evaluation of the Impact of the Clinical Periodontal Status on Volumetric Features of Gingival Crevicular Fluid by using Periotron® 8000. *Journal of Hard Tissue Biology*. 2017;26(2):187-94.
  20. Turesky S, Gilmore, Glickman : Reduced plaque formation by the chloromethyl analogue of vit C. *J Periodontol* 1970;41:41-49.
  21. Muhlemann. HR: Psychological and chemical mediators of gingival health .*J Prevodent* 1977;4:6-17.
  22. Chandak LG, Lohe VK, Bhowate RR, Gandhi KP, Vyas NV. Correlation of mandibular radiomorphometric indices with serum calcium and serum estradiol in pre-and post-menopausal women. *Contemporary clinical dentistry*. 2017 Jan;8(1):53.
  23. Simon BI, Zatzoff AL, Kong JW, O Connell S.M. Clinical and histological comparison of extraction socket healing following the use of autologous platelet-rich fibrin matrix (PRFM) to ridge preservation procedures employing demineralized freeze dried bone allograft material and membrane. *Open Dent J* 2009;3:92-9.
  24. Garg S, Arora SA, Chhina S, Singh P. Multiple gingival recession coverage treated with vestibular incision subperiosteal tunnel access approach with or without platelet-rich fibrin - A case series. *Contemp Clin Dent* 2017;8:464-8.
  25. Agrawal, D.R., and P.G. Jaiswal. "Injectable Platelet Rich Fibrin (I-PRF): A Gem in Dentistry." *International Journal of Current Research and Review* 12, no. 21 (2020): 25–30. <https://doi.org/10.31782/IJCRR.2020.122116>.
  26. Deshpande, Aniruddha, Somnath B. Koudale, and Manohar L. Bhongade. "A Comparative Evaluation of RhPDGF-BB plus Beta-TCP and Subepithelial Connective Tissue Graft for the Treatment of Multiple Gingival Recession Defects in Humans." *INTERNATIONAL JOURNAL OF PERIODONTICS & RESTORATIVE DENTISTRY* 34, no. 2 (April 2014): 241–49. <https://doi.org/10.11607/prd.1726>.
  27. Thombre, Vivek, Somnath B. Koudale, and Manohar L. Bhongade. "Comparative Evaluation of the Effectiveness of Coronally Positioned Flap With or Without Acellular Dermal Matrix Allograft in the Treatment of Multiple Marginal Gingival Recession Defects." *INTERNATIONAL JOURNAL OF PERIODONTICS & RESTORATIVE DENTISTRY* 33, no. 3 (June 2013): E88–94. <https://doi.org/10.11607/prd.1371>.

#### REVIEWER COMMENT ??

- Q1. Modification in selection and arrange the key words ?
- Q2. Which radiographic view/projection for bone quality/quantity evaluation??
- Q3. Kindly specify about word planning?
- Q4. Type of suture materials Absorbable / Unabsorbable ??
- Q5. Brand name of drug to be used does not match with molecule composition ?
- Q6. Reframe the journal title name ?