ORIGINAL RESEARCH

A clinical and Radiographical evaluation of PRF vs Chorion membrane with DFDBA in Grade II Furcation defects

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ABSTRACT

Introduction: Treatment of molar furcation defects remains a considerable challenge in clinical practice. Furcation defects treatment can vary according to the type and location of the defects. Regenerative procedures are one of the treatment modalities in furcation involvement. **Objectives:** The aim of this study designed to comparatively evaluate the efficacy of platelet rich fibrin membrane and chorion membrane with bone graft (Demineralised Freeze Dried Bone Allograft) in the management of grade II furcation defects. **Materials and methods:** This study was conducted clinically on fortysites with grade II furcation defects. Patients were divided into two groups: Group 1 PRF And DFDBA and Group 2 included Chorion Membrane and DFDBA. Vertical Furcation Depth, Horizontal Fucration depth, relative attachment level, were measured at baseline,3, 6 and 9 months . Radiographic examination was performed at baseline,6, and9months to evaluate bonefill in the furcation defect. **Results and Conclusion:** There was a significant reduction of clinical parmeters and defect fill with autogenous PRF and Chorion membrane with DFDBA in Grade II furcation defects

Keywords: Amniotic chorion membrane- Grade II furcation defects, platelet rich fibrin. A clinical and Radiographical evaluation of PRF vs Chorion membrane with DFDBA in Grade II Furcation defects. A 9 month study

INTRODUCTION

Periodontitis is an inflammatory disease that destroys the periodontium, including the alveolar bone, and if left untreated, can lead to tooth loss. It affects the supporting tissues of the teeth and alters the morphology of bone resulting in various types of osseous defects like horizontal bone loss, vertical or angular bone defects, osseous craters, bulbous bone contours, ledges, reversed architecture and furcation involvement.

Furcation involvement is defined as the extension of inflammatory periodontal disease into the interradicular area of multirooted teeth. It involves pathologic resorption of bone and damage to periodontal ligament fibers in the bifurcation and trifurcation areas of teeth[1].

The goals of conventional periodontal therapy, both non surgical and surgical, are aimed at improving the health of the periodontal tissues and at arresting the periodontal disease, but this therapy does not replace the lost tissues. Glickman in 1953 classified furcation defects into Grade I, II, III and IV depending on the amount of tissue destruction. Furcation-involved molar teeth respond less favourably to conventional periodontal therapy, and molars are lost more often than any other tooth type. Access to the furcation areas is complicated by the posterior location of the molars, the disparity between root and furcation anatomies, and the shape and dimension of the debriding instruments[3]. Root debridement is consequently very difficult and inefficient in furcations.

Therapies range from thorough debridement to regenerative procedures and, if the lesion progresses, to extraction. Roughly over the past 20 years, the outcomes have changed in part because of the new knowledge about the disease process and wound healing, and in part because of the availability of new materials.

The principle of GTR was promulgated in 1982 by Nyman et al for treatment of osseous defects in human periodontitis[5]. GTR has been shown to be useful in the treatment of grade II furcation lesions. Some authors suggest that the use of bone substitution materials (either as having osteoconductive properties or just as providers of support for the membranes to prevent their collapse over the furcation defect) improves the outcome of this type of treatment, while others report no improvement over the use of surgical membranes alone. However, GTR in combination with various types of bone grafts remains one of the most successful surgical regenerative therapies for grade II furcation defects[6].

Various Barrier Membranes used in regeneration have been classified as non-bioresorbable and bioresorbable. Non bioresorbable membranes include polytetrafluoroethylene membrane (Goretex), Teflon membrane and Millipore filters. Bioresorbable membranes include rat, bovine or porcine collagen membrane, cargile membrane, polylactic acid, vicryl (polyglactin 910), synthetic skin (biobrane), freeze dried durameter, Platelet Rich Fibrin (PRF) membrane, amnion membrane and chorion membrane. Bioresorbable membranes are gaining popularity in guided tissue regeneration therapy as they are resorbed within the body and there is no need for second surgery for removal of membrane.

Platelet-rich fibrin membrane (PRF), a second generation platelet concentrate introduced by Dohan DM *et al (*2006) is a matrix of autologous fibrin, in which a large quantity of platelet and leukocyte cytokines are embedded during centrifugation leading to their progressive release over time. Platelet Rich Fibrin has a fibrin network similar to natural one that leads to effective cell migration and proliferation during healing process [7].

Chorion membrane which possesses mesenchymal stromal cells has the property of stemness, self renewal and hierarchy. Due to the differentiation potential of chorionic mesenchymal stromal cells towards classic mesodermal lineages it can be used for clinical applications in transplantation and tissue regeneration[8].

The present study was therefore designed to comparatively evaluate the efficacy of platelet rich fibrin membrane and chorion membrane with bone graft (Demineralised Freeze Dried Bone Allograft) in the management of grade II furcation defects.

MATERIALS AND METHODS

A clinical and radiographical study was carried in 40 sites in patients reporting to Out Patient Department (OPD) of Periodontology of Indira Gandhi Govt Dental College Jammu suffering from moderate to severe periodontitis with clinical and radiographical evidence of grade II furcation defects of aged 20-50 years. Smokers, pregnant and lactating women and those who didn't fulfil the inclusion criteria were excluded from the study.

MATERIALS USED IN THE STUDY

1. BONEGRAFT

a) Demineralised freeze-dried bone allograft (DFDBA): The DFDBA of size < 500µm was obtained from Tata Memorial Hospital Tissue Bank, Mumbai, India.

2. GTR MEMBRANES

a) Platelet Rich Fibrin (PRF) membrane: The protocol for PRF membrane formation was performed according to the Choukroun's procedure. 10 ml of intravenous blood (by venipuncturing of the antecubital vein) was collected in a sterile tube without anticoagulant, followed by an immediate centrifugation at 3000 rpm, for 10 minutes.

b) Chorion membrane (CM): The CM 3 x 3 cm was obtained from, Tata Memorial Hospital Tissue Bank, Mumbai, India.

CLINICAL AND RADIOGRAPHICAL PARAMETERS

Clinical parameters: Vertical probing depth and relative attachment level were measured using UNC-15 Periodontal probe and horizontal probing depth was measured using color coded Nabers probe at baseline, 3 months, 6 months and 9 months after the surgery.

All the measurements were standardized using customized acrylic stents with grooves, which were prepared on the study model of the patients. The occlusal stents were made using cold cure pink acrylic.

Vertical Probing Depth was measured from gingival margin (GM) to the base of the pocket (BOP) at furcation area.

Horizontal probing depth was measured at furcation area from fixed reference point (apical end of stent).

Relative attachment level was measured from fixed reference point (apical end of stent) to the base of pocket at furcation area.

2. Radiographic parameters: Radiographic defect fill was measured using Intraoral periapical radiographs (IOPA) using long cone/paralleling technique. The radiographs were standardized using Rinn-XCP (extension cone paralleling) instrument and a radiographic grid calibrated in millimetres. The distance was measured by counting the millimetre markings on the radiograph. The radiographs were taken at the baseline, 3 months, 6 months and 9 months post-operatively. The vertical distance between the fornix and the base of the defect was recorded from radiographs.

SURGICAL PROCEDURE

Premedication was given to the patients before the surgery. All patients were made to rinse with 0.2% chlorhexidine mouthrinse for 60 seconds, prior to the surgery. Local anaesthesia was obtained using block and infiltration techniques with lignocaine 2% with adrenaline 1:2,00,000.. The crevicular incisions were given at the defect site, extending one tooth adjacent to the involved tooth, both mesially and distally using a Bard-Parker handle and surgical blade no. 11, 12 and 15. A full thickness mucoperiosteal flap was reflected to provide access to the furcation defect and surrounding alveolar bone. The defect was thoroughly debrided with the help of area specific gracey curettes to obtain a smooth hard surface. Pre-suturing of the flap was done and the sutures were left loose for the insertion of the graft and membrane.

In Group I, DFDBA bone graft, was placed to fill the defect and was covered with PRF membrane and sutured followed by application of periodontal dressing (Coe-Pack).

In group II, DFDBA was placed to fill the defect and was covered with Chorion membrane and sutured followed by application of periodontal dressing (Coe-Pack). The patients were given post-surgical instructions and medications.

STATISTICAL ANALYSIS

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as Mean±SD. Wilcoxon Signed Ranks Test and Mann-Whitney Test was employed for comparing continuous variables.

RESULTS

Clinical and radiographic measurements were recorded at baseline, 3 months, 6 months and 9 months postoperatively. The recorded data was put to statistical analysis and the results obtained were compared.

VERTICAL PROBING DEPTH

The mean VPD at baseline for Group I was 4.666 ± 1.154 mm, at 3 months 2.833 ± 0.717 mm, 6 months 2.750 ± 0.621 mm, and at 9 months was 2.666 ± 0.651 mm. The mean VPD at baseline for Group II was 4.750 ± 1.484 mm, at 3 months 2.333 ± 0.492 mm, at 6 months 2.416 ± 0.792 mm, and at 9 months was 2.833 ± 0.834 mm.).(Table I)

Wilcoxon Signed Ranks Test was performed to analyze the changes in VPD at different time intervals within the same group. Group I showed statistically highly significant change in VPD from baseline to 3 months (p = 0.002), baseline to 6 months (p = 0.002), baseline to 9 months (p = 0.002), (Table 1) whereas the results were statistically not significant from 3 to 6 months (p = 0.317), 3 to 9 months (p = 0.157) and 6 to 9 months (p = 0.317).

Group II, showed statistically highly significant change in VPD from baseline to 3 months (p = 0.002), baseline to 6 months (p = 0.002), baseline to 9 months (p = 0.002), (Table 1) however significant change was shown from 6 to 9 months (p = 0.025) whereas the results were statistically not significant from 3 to 6 months (p = 0.655) and 3 months to 9 months (p = 0.084)

Mann-Whitney Test was performed to compare the changes in VPD, in Group I and Group II at baseline and at various time intervals. At baseline p value was 0.976, 3 months was 0.071, 6 months was 0.242 and 9 months was 0.662.

Table 1: Mean and Standard Deviation of vertical probing depth

Vertical Probing Depth	Base line (Mean ± SD)	3 months (Mean ± SD)	6 months (Mean ± SD)	9 months (Mean ± SD)
Group I	4.666 ± 1.154	2.833 ± 0.717	2.750 ± 0.621	2.750 ± 0.621
P value (From baseline)		0.002*	0.002*	0.002*
Group II	4.750 ± 1.484	.333 ± 0.492	2.416 ± 0.792	2.833 ± 0.834
P value (From baseline)		0.002*	0.002*	0.002*

*Statistical Significance p≤0.05

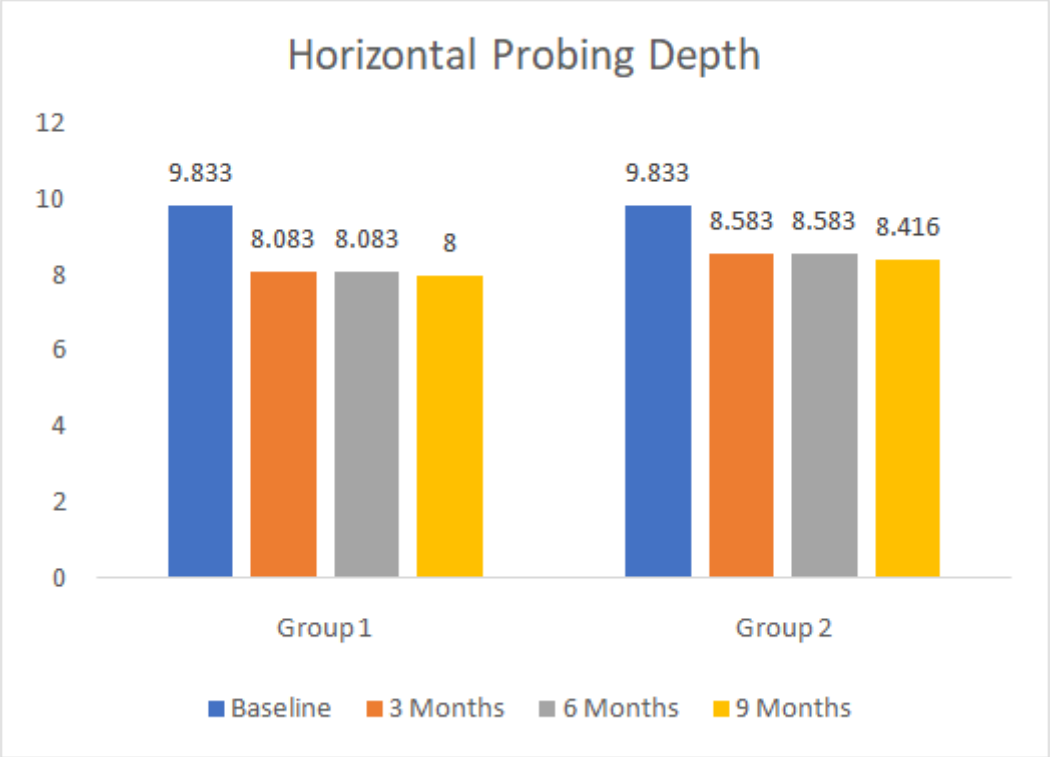
HORIZONTAL PROBING DEPTH

The mean HPD at baseline for Group I was 9.833 ± 1.527 mm, at 3 months 8.083 ± 1.378 mm, 6 months 8.083 ± 1.378 mm, and at 9 months was 8.000 ± 1.414 mm. The mean HPD at baseline for Group II was 9.833 ± 2.249 mm, at 3 months 8.583 ± 2.391 mm, at 6 months 8.583 ± 2.108 mm, and at 9 months was 8.416 ± 1.975 mm. (Graph 1)

Wilcoxon Signed Ranks Test was performed to analyze the changes in VPD at different time intervals within the same group. Group I showed statistically highly significant change in VPD from baseline to 3 months (p = 0.002), baseline to 6 months (p = 0.002), baseline to 9 months (p = 0.002), whereas the results were statistically not significant from 3 to 6 months (p = 0.317), 3 to 9 months (p = 0.157) and 6 to 9 months (p = 0.317).

Group II, showed statistically highly significant change in VPD from baseline to 3 months (p = 0.002), baseline to 6 months (p = 0.002), baseline to 9 months (p = 0.002), however significant change was shown from 6 to 9 months (p = 0.025) whereas the results

were statistically not significant from 3 to 6 months (p = 0.655) and 3 months to 9 months (p = 0.084)
Mann-Whitney Test was performed to compare the changes in VPD, in Group I and Group II at baseline and at various time intervals. At baseline p value was 0.976, 3 months was 0.071, 6 months was 0.242 and 9 months was 0.662.



RAL- RELATIVE ATTACHMENT LEVEL
The mean RAL, at baseline for Group I was 9.250 ± 1.764 mm, at 3 months 8.333 ± 1.435 mm, 6 months 8.000 ± 1.595 mm and at 9 months 7.833 ± 1.337 mm. The mean RAL at baseline for Group II was 9.250 ± 2.416 mm, at 3 months 7.916 ± 2.274 mm, at 6 months 8.250 ± 2.378 mm and at 9 months 8.333 ± 2.146 mm.
For comparison of the change between two groups, t-test was performed. It shows the comparison of means of the change in RAL. The results were significant for Group I in comparison to Group II at 3 to 9 months (0.019) however the change was not statistically significant at baseline to 3 months (p = 0.438), baseline to 6 months (p = 0.740), baseline to 9 months (p = 0.328), 3 to 9 months (p = 0.206) and 6 to 9 months (p = 0.192).

RADIOGRAPHIC CHANGES
The Mean for Group I at baseline was 2.333 ± 1.073 mm, at 3 months 1.833 ± 1.114 mm, 6 months 1.000 ± 0.953 mm, 9 months 0.833 ± 0.937 mm. The mean RAL at baseline for Group II was 2.583 ± 0.668 mm, at 3 months 1.666 ± 0.651 mm, at 6 months 1.166 ± 0.834 mm and at 9 months 0.583 ± 0.792 mm.
Table IV shows the comparison of means of change in radiographic measurements from fornix to base of defect for both groups at different time intervals. The results were statistically significant for Group I in comparison to Group II at baseline to 3 months (p = 0.038), but not significant at baseline to 6 months (p = 0.974), baseline to 9 months (p = 0.159), 3 to 6 months (p = 0.229), 3 to 9 months (p = 0.572) and 6 to 9 months (p = 0.159) for the means of the change in radiographic measurements from fornix to base of defect

DISCUSSION
The ultimate objective of periodontal therapy is to regenerate tissues lost as a consequence of periodontal disease. Various treatment modalities for treating furcation defects involve either maintaining existing furcation (scaling and root planing), increasing access to furcation (gingivectomy/ apically positioned flap, odontoplasty, osteoplasty/ostectomy), or elimination of furcation (root amputation/ tooth resection, bicuspidization)[10].
The newer aspect involves the utilization of regenerative procedures for the treatment of furcation defects which includes placement of bone grafts and the use of organic or synthetic barrier membranes based on the principle of guided tissue regeneration (GTR)[10].
Guided tissue regeneration is a successful procedure for class II furcation defects as indicated in studies by Pontoriero R et al (1987)[11], Lekovic V et al (1989)[12], Caffesse R et al (1990)[4] and Mellonig J et al (1994)[13] who demonstrated greater pocket depth improvement

and clinical attachment gain in class II furcation defects treated with ePTFE membranes vs control sites of open flap debridement.

Bioresorbable barriers were developed to overcome the disadvantages of the nonresorbable barriers. Gottlow J et al (1994)[14] demonstrated histologically new attachment, new cementum, and bone at 6 weeks in the experimental monkeys with the barrier being stable and there was no inflammation.

The barrier membranes recommended for use in GTR, regardless of the material used, must be safe, biocompatible, non-toxic, not induce any inflammatory response, and be designed for clinical applicability based on the morphology of the osseous defects[15]. One of the oldest biomaterials used for scaffolds is the foetal membrane. The foetal membrane was first used for the transplantation of skin in 1910. It is useful in the management of burns; creation of surgical dressings; as well as reconstruction of oral cavity, bladder and vagina; tympanoplasty; and arthroplasty. In addition, the chorionic membrane (CM) a foetal membrane is abiomaterial that can be easily obtained, processed andtransported[16].

It has been found that PRF consists of a fibrin matrix polymerized in a tetramolecular structure; the incorporation of platelets, leukocyte, and cytokines; and circulating stem cells. Slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes. In addition, PRF slows down the blood activation process, which could induce an increased leukocyte degranulation and cytokine production from proinflammatory mediators, such as interleukin (IL)-1 β, IL-6, and tumor necrosis factor-α, to anti-inflammatory cytokines, such as IL-4. It is also found that PRF organizes as a dense fibrin scaffold with a high number of leukocytes concentrated in one part of the clot, with a specific slow release of growth factors (e.g., transforming growth factor-1β, PDGF-α,β and vascular endothelial growth factor) and glycoproteins (e.g., thrombospondin-1) during < 7 days[17].

Presently, however, there are very few studies on use of PRF with DFDBA and chorion membrane with DFDBA in management of grade II furcations defects. Therefore, in the present study, an attempt has been made to evaluate and compare, clinically and radiographically the regenerative potential of autologous PRF membrane with DFDBA and chorion membrane with DFDBA in the treatment of grade II mandibular furcations defects in moderate to severe periodontitis patients.

Pocket depth reduction by PRF membrane and DFDBA was reported by Lafzi A et al (2012)[19], Kaul S et al (2012)[10], Piemontese M et al (2008)[20] and Bansal C and Bharti V (2013)[25]. This reduction may be attributed to beneficial effects of PRF. PRF consists of a fibrin matrix polymerised in a tetramolecular structure; the incorporation of platelets, leukocytes, and cytokines; and circulating stem cells. Slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycan chains in the fibrin meshes. PRF is able to progressively release cytokines during fibrin matrix remodeling. It is also found that PRF organizes as a dense fibrin scaffold with a high number of leukocytes concentrated in one part of the clot. It is an optimal matrix for migration of endothelial cells and fibroblasts. It permits a rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue. Such a mechanism might explain the clinically observed soft tissue healing properties of PRF[8].

In Group II, The reduction in VPD was in accordance with studies such as Kothiwale SV (2013)[16], Holtzclaw DJ (2012)[21], Rosen PS (2011)[23] and Suresh DK and Gupta A (2012) [26] This may be credited for the presence of tissue inhibitor of metalloproteinases (TIMPs) in chorionic membrane which suppresses matrix metalloproteinases (MMPs) and transforming growth factor beta (TGF- β) which stimulates the production of TIMPs from the surrounding tissue. As epithelial cells quickly migrate across the CM barrier, they form a seal over the underlying bone graft and do not apically migrate into the defect[3].

Both the groups showed significant reduction in vertical probing depth that was in agreement with studies by Wang HL et al (1994)[22], Garret S et al (1997)[27] and Luepke PG et al (1997)[24.]This reduction is attributed to the combination of a GTR membrane with bone graft, which resulted in better coronal extension of the portion of the wound that healed with complete periodontal regeneration[12].

On intergroup comparison the improvement shown by Group I in comparison to Group II was not statistically significant at 3, 6 and 9 months of the study.

Horizontal Probing Depth (HPD) The HPD reduction was statistically highly significant (p = 0.003) from baseline to 9 months. This was in accordance with studies done by Lafzi A et al (2012)[19], Luepke PG et al (1997)[24], Kaul S et al (2012)[10], Piemontese M et al (2008)[20], and Bansal C and Bharti V (2013)2[8]. This supports the role of various growth factors present in the PRF in accelerating the soft and hard tissue healing, along with retaining the DFDBA in the furcation defects and providing the spatial relationship for bridging the graft material with the vascular and cellular elements from the periodontal ligament and adjacent osseous wall[62].

In Group II, The reduction in HPD was in accordance with studies such as Kothiwale SV (2013)[16], Holtzclaw DJ (2012)[21]and Suresh DK and Gupta A (2012)[26]. The placement of chorion membrane over the graft reduced the likelihood of exposure to the oral environment during the period of postoperative wound healing. Chorion barrier not only contains the graft but also prevents the ingrowth of connective tissue into the future developing attachment apparatus

When the change in HPD was compared between two groups the improvement shown by Group I in comparison to Group II was not statistically significant suggesting that both treatment modalities are comparable.

Relative Attachment Level (RAL) – The change in RAL following regenerative therapy is the single most commonly used clinical outcome variable in regenerative studies.

This is in accordance with the studies done by Lafzi A et al (2012)[19], Luepke PG et al (1997)[24], Kaul S et al (2012)[10], Piemontese M et al (2008)[20], Kanakamedala A et al (2009)[48] and Bansal C and Bharti V (2013)[25]. The gain in relative attachment level may be attributed to the benefitial effects of PRF membrane.

Holtzclaw DJ (2012)[40], gain in relative attachment level corresponds that collagen layers of chorion are rich in collagen type I, IV, V, VI, proteoglycans, laminin and fibronectin. Fibronectin is involved in many cellular processes including tissue repair, blood clotting, cell migration and adhesion.

When the change in RAL was compared between two groups; the improvement shown by Group I in comparison to Group II was not statistically significant.

Radiographic Bone Fill (from fornix to base of defect) – The mean fornix to base of defect distance. This is in accordance with the studies done by by Lafzi A et al (2012)[19], Luepke PG et al (1997)[24], Kaul S et al (2012)[10], Piemontese M et al (2008)[20] and Bansal C and Bharti V (2013)[25]. The combination of DFDBA and PRF membrane provided benefit of rapid and early bone formation. The ability of demineralized bone to induce new bone formation in soft tissues and to enhance bone formation in osseous tissues is believed to be due to the content and diffusibility of bone morphogenetic proteins (BMPs) present in the material. The BMPs and other growth factors and cytokines interact with mesenchymal stem cells or undifferentiated osteogenic precursors in the host tissue, causing them to differentiate into bone-forming cells[24]. Several other growth factors like FGF (fibroblast growth factor), IGF-I (insulin-like growth factor-I), TGF-β1 (transforming growth factor- beta1), VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor) are also identified.

Nevins et al (2003)[30] revealed a robust regeneration of a complete new attachment apparatus, including bone, periodontal ligament and cementum in human interproximal intrabony defects and molar class II furcation lesions following the application of purified recombinant human platelet- derived growth factor BB (rhPDGF-BB) incorporated in demineralized freeze-dried bone allograft (DFDBA).

The increases in the radiographic bone fill can be attributed to DFDBA and Chorion Membrane that act as a barrier membrane between the gingival epithelium and hard tissue to promote the periodontal ligament cells to form progenitor cells that can regenerate new tissues. Often the graft site must bear loads at or close to physiologic levels very soon after transplantation. CM when used as internal fixation provides this early stability. The barrier is placed dry and quickly hydrates with blood and becomes very pliable, and closely adapts to the contours of the underlying surface.

A high rate of furcation fill with GTR combined with a bone graft material in both the groups is in accordance with the study done by Schallhorn RG and McClain PK (1988)[29] who reported complete defect fill in 72% cases of class II and III furcations treated with GTR membrane and DFDBA.

Thus, within the limitations of present study both the treatment modalities, PRF membrane with DFDBA and Chorion membrane with DFDBA showed a definite but comparable improvement in all the clinical and radiographic parameters. However, assessment of periodontal regeneration requires histological evidence and surgical re-entry which could not be carried out in the present study because of ethical considerations and patient management limitations

Therefore, further studies with higher number of subjects, long term observations and histological evaluation should be done.

REFERENCES

1. Jaiswal R, Deo V. Evaluation of the effectiveness of enamel matrix derivative, bone grafts, and membrane in the treatment of mandibular class ii furcation defects.*Int J Periodontics Restorative Dent. 2013; 33:58–64.*
2. Rock L.Potential of platelet rich fibrin in regenerative periodontal therapy: literature review.*Can J Dent Hygeine. 2013; 47 (1): 33–37.*
3. Khanna D, Malhotra S, Naidu DV. Treatment of grade II furcation involvement using resorbable guided tissue regeneration membrane: A six-month study. *J Ind Soc Periodontol. 2012; 16(3): 404–410.*
4. Caffesse RG, Smith BA, Duff B, Morrison EC, Merrill D, Becker W. Class II furcations treated by guided tissue regeneration in humans: case reports. *J Periodontol.1990; 61; 510–514.*
5. Nyman S, Lindhe J, Karring T, Rylander H. New attachment following surgical treatment of human periodontal disease. *J Clin Periodontol. 1982; 9: 290–296.*
6. Marinescu AG, Carligeriu V, Nica L, Onisei D, Rusu D, Boariu M, Epistatu D, Cioca D, Stefan-Ioan Stratul SL. Evaluation of treatment techniques for advanced (grade II-IV) furcation defects. A literature review of the regenerative methods.*TMJ 2011;61.1(2):115-123*
7. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet related biologic features.*Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(3):45–50.*
8. Parolini O, Alviano F, Bagnara GP, Bilic G, Bühring HJ, Evangelista M, Hennerbichler S, Liu B, Magatti M, Mao N, Miki T, Marongiu F, Nakajima H, Nikaido T, Portmann-Lanz CB, Sankar V, Soncini M, Stadler G, Surbek D, Takahashi TA, Redl H, Sakuragawa N, Wolbank S, Zeisberger S, Zisch A, Strom SCConcise Review: Isolation and characterization of Cells from

Human Term Placenta: Outcome of the First International Workshop on Placenta Derived Stem Cells.*Stem Cells. 2008; 26(2):300-11.*

9. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution.*Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(3):37–44.*
10. Kaul S, Reddy S, Prasad MG, Asutkar H, Kumari S, Agnihotri J, Amudha Sharma S. Management of mandibular furcation defects using platelet rich fibrin-a case series. *Int J Dent Case Reports 2012; 2(2): 58-64.*
11. Pontoriero R. Nyman S, Lindhe J, Rosenberg E, Sanavi F. Guided tissue regeneration in the treatment of furcation defects in man. *J Clin Periodontol. 1987; 14: 618-620*
12. Camelo MC, Neuins ML, Nevins M. Treatment of Class II Furcations with Autogenous Bone Grafts and e-PTFE Membranes. *Int J Periodontics Restorative Dent. 2000; 20:233-243.*
13. Mellonig JT, Seamons BC, Gray JL, Towle HJ. Clinical evaluation of guided tissue regeneration in the treatment of grade II molar furcation invasions. *Int J Periodontics Restorative Dent.1994; 14: 255–271.*
14. Gottlow J, Laureil L, Lundgren D, Mathisen T, Nyman S, Rylander H, Bogentoft C. Periodontal tissue response to a new bioresorbable guided tissue regeneration device: a longitudinal study in monkeys. *Int J Periodontics Restorative Dent.1994; 14:436-449.*
15. Gottlow J, Nyman S, Karring T, Lindhe J. New attachment formation as the result of controlled tissue regeneration. *J Clin Periodontol. 1984:11:494-503.*
16. Kothiwale SV. The clinical evaluation of chorionic membrane in guided tissue regeneration for periodontal pocket therapy: a clinical and radiographic study. *Cell Tissue Bank. 2013 Jul 10.*
17. Bajaj P, Pradeep AR, Agarwal E, Rao NS, Naik SB, Priyanka N, Kalra N. Comparative evaluation of autologous platelet-rich fibrin and platelet-rich plasma in the treatment of mandibular degree II furcation defects: a randomized controlled clinical trial. *J Periodont Res. 2013; 48: 573–581.*
18. Axelsson P, Nystrom B, Lindhe J. The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. *J Clin Periodontol. 2004; 31: 749–757.*
19. Lafzi A, Shirmohammadi A, Faramarzi M, Jabali S, Arman Shayan. Clinical Comparison of Autogenous Bone Graft with and without Plasma Rich in Growth Factors in the Treatment of Grade II Furcation Involvement of Mandibular Molars. *J Dent Res Dent Clin Dent Prospect. 2013; 7(1):22-29.*
20. Piemontese M, Aspriello DM, Rubini C, Ferrante L, Procaccini M. Treatement of intrabony defects with demineralized freeze dried bone allograft in combination with platelet rich plasma: a comparative clinical trial. *J Periodontol. 2008; 79:80-810.*
21. Hamp SE, Nyman S, Lindhe J. Periodontal treatment of multirooted teeth. *J Clin Periodontal.1975:2; 126-135.*
22. Butler JR, Rajnay ZW, Vernino AR, Parker D. Volumetric changes following barrier regeneration procedures for the surgical management of grade m molar furcation defects in baboons: II. bone, cementum, epithelium, and connective tissue.*Int J Periodontics Restorative Dent. 1998; 18:59-69.*
23. Stahl SS, Froum S, Tarnow D. Human histologic responses to guided tissue regenerative techniques in intrabony lesions. Case reports on 9 sites. *J Clinical Periodontol.1990; 17: 191–198.*
24. Luepke PG, Mellonig JT, Brunsvold MAA. Clinical evaluation of a bioresorbable barrier with and without decalcified freeze-dried bone allograft in the treatment of molar furcations.*J Clin Periodontol. 1997; 24(6):440-6.*
25. Bansal C,Bharti V. Evaluation of efficacy of autologous platelet-richfibrin with demineralised-freeze dried bone allograft in the treatment of periodontal intrabony defects. *J Indian Soc Periodontol. 2013; 17(3):361-366.*
26. Suresh DK, Gupta A. Gingival Biotype Enhancement and Root Coverage Procedure. *Clin Adv Periodontics 2013; 3:237-242.*
27. Garrett S, Martin M, Egelberg J. Treatment of periodontal furcation defects. Coronally posioned flaps versus duramater membranes in class II defects. *J Clin Periodontol.1997; 17: 179–185.*
28. Garrett S. Periodontal regeneration around natural teeth. *Annals of Periodontol. 1996; 11: 621–666.*
29. Schallhorn RG, McClain PK. Combined osseous composite grafting, root conditioning, and guided tissue regeneration.*Int J Periodontics Restorative Dent. 1988; 8 (4):8-31.*
30. Nevins M, Camelo M, Nevins ML, Schenk RK, Lynch SE.
Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone.*J Periodontol. 2003; 74(9):1282-92.*