

## Evaluation of Regeneration in Mandibular Grade II Furcation Defects Treated with Autologous Platelet Rich Fibrin and its Comparison with Heliguide and DFDBA: A Randomized Controlled Clinical Trial

Shreya Joshi<sup>1</sup>, Pradeep Kumar<sup>2</sup>, Rohan Gupta<sup>3</sup>, Sonia Godara<sup>4</sup>, Rajendra Yadav<sup>5</sup>, Garima<sup>6</sup>

<sup>1</sup> MDS Resident, Department Of periodontics, Rajasthan Dental College and Hospital, Jaipur, Rajasthan, India

<sup>2</sup> Professor and Head, Department Of periodontics, Rajasthan Dental College and Hospital, Jaipur, Rajasthan, India

<sup>3</sup> Associate Professor, Department Of periodontics, Rajasthan Dental College and Hospital, Jaipur, Rajasthan, India

<sup>4</sup> Assistant Professor, Department Of periodontics, Rajasthan Dental College and Hospital, Jaipur, Rajasthan, India

<sup>5</sup> Associate Professor, Department Of periodontics, Rajasthan Dental College and Hospital, Jaipur, Rajasthan, India

<sup>6</sup> PG Resident, Department Of periodontics, Rajasthan Dental College and Hospital, Jaipur, Rajasthan, India

---

Received: 22-02-2022 / Revised: 26-03-2022 / Accepted: 27-04-2022

Corresponding author: Dr. Rajendra Yadav

Conflict of interest: Nil

---

### Abstract

**Background:** To evaluate periodontal regeneration in Mandibular grade II furcation defects following treatment with Platelet rich fibrin compared to demineralized freeze- dried bone allograft and GTR membrane.

**Materials And Methods:** A total of 20 systemically healthy patients undergoing periodontal therapy will be included and with bilateral buccal grade II furcation defect in the mandibular molars, participated in the study.

The following clinical measurements were recorded at baseline as well as 6 months post-surgery: (1) Plaque Index, (2) Sulcus bleeding index (SBI), (3) Probing depth from the gingival margin, relative vertical clinical attachment level (RVCAL). Paired student's t-test was done to test the significance of changes overtime from baseline to 6 months. The unpaired t-test was utilized to compare intergroup changes in parameters. Comparison were also drawn between the test and the control groups by applying the Paired "t" test, unpaired "t" test and Chi-square test.

**Results:** The mean gain in relative clinical attachment levels in the test and control groups, at the end of six months, the mean gain in the vertical clinical attachment level was  $1.30 \pm 0.67$  mm (Test group) &  $1.70 \pm 0.67$  mm (control group). The mean gain in the horizontal clinical attachment level was  $1.80 \pm 3.35$  mm (Test group) &  $1.60 \pm 1.5$ mm (control group). The mean probing pocket depth for the control group at baseline was  $5.50 \pm 0.52$  mm whereas values after 6 months post-surgery was  $2.60 \pm 0.51$  mm.

**Conclusion:** Both treatment modalities demonstrated a significant improvement in the probing depth, vertical and horizontal relative attachment level as well as radiographic bone density at 6 months surgery. There was no significant difference in the result obtained from the use of both the treatment modalities. Hence further long-term studies are requiring

substantiating the efficacy of cost effective PRF in the treatment of mandibular grade II furcation defects over other GTR membranes which are presently being used.

**Keywords:** GTR, Grade II furcation, PRF and bone grafts

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

## Introduction

Periodontitis is a disease of multifactorial origin, “an inflammatory disease of the teeth caused by specific microorganisms or group of microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both”. Progression of loss of attachment horizontally into the region between roots creates an additional problem of access and leads to furcation involvement. Depending upon the severity of involvement furcation has been classified into grade I, grade II, grade III and grade IV. [1] Grade II furcation lesion is a cul-de-sac with a definite horizontal component. Surgical access significantly enhances removal of calculus from molars with furcation invasion. The primary objective of any furcation therapy is the elimination of the pocket by respective or regenerative procedures and making the area accessible for plaque control. The main objective of furcation treatment is to eliminate microbial plaque and to establish a proper tooth contour to facilitate self-performed plaque control. Various treatment modalities have been proposed to improve the prognosis of furcation involvement. [2] Furcations are generally less responsive to therapy than non-furcated areas and/or single rooted teeth, as reflected by a limited gain in attachment levels, less reduction in probing depths (PDs), more frequent bleeding on probing (BOP), and higher microbiologic counts after conventional non-surgical and open flap instrumentation of furcation surfaces. Multiple approaches have been used to resolve furcation defect including autografts, demineralized freeze-dried bone allografts (DFDBAs), bovine-derived xenografts, barrier membranes, and

combinations of membranes and bone grafts. [3]

## Material and methods

A total of 20 patients, 10 females and 10 males, in the age group of 18-65 years with bilateral buccal grade II furcation defects in mandibular molars participated in the study. The criteria for selection were Patients with grade II furcation defects in Mandibular molars on buccal side with radiolucency in furcation area in an intraoral periapical radiograph, patient's age  $\geq$  25yrs, probing depth  $>$  4mm, horizontal probing depth  $>$ 2mm after phase I therapy.

The subjects received detailed information regarding their condition and the treatment plan. Oral hygiene instructions were given. After being informed about the aim of the project, a signed consent was taken from the patient.

## Clinical data collection-

The following clinical measurements were recorded at baseline as well as six months post-surgery-Plaque Index (PI), Sulcus bleeding index (SBI), Probing depth from the gingival margin, Relative vertical clinical attachment level (RVCAL), Relative Horizontal clinical attachment level (RHCAL) along with gingival margin level (GML).

One site in each patient was randomly allocated to the control group and was treated by GTR and DFDBA allograft. The GTR membrane used was Healiguide. The contralateral site test group which was treated using Platelet rich fibrin (PRF) alone.



**Figure 1: Control site 46 (A) pre operative, -PPD (from gingival margin to the base of the pocket, VCAL (from fixed reference point to the base of pocket) and GML (from fixed reference point to the gingival marginal level).**

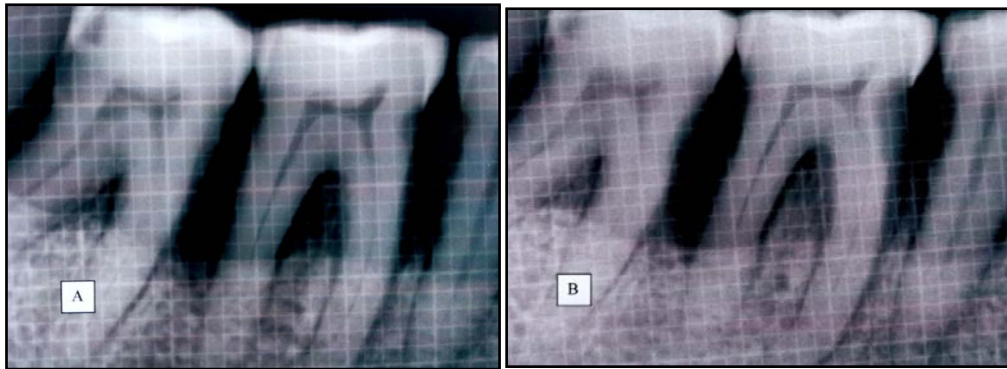


**Figure 2: Test site in 37 (a) pre operative and PPD (from gingival margin to the base of the pocket), VCAL (from fixed reference point to the base of the pocket) and GML (from fixed reference point to the gingival margin)**

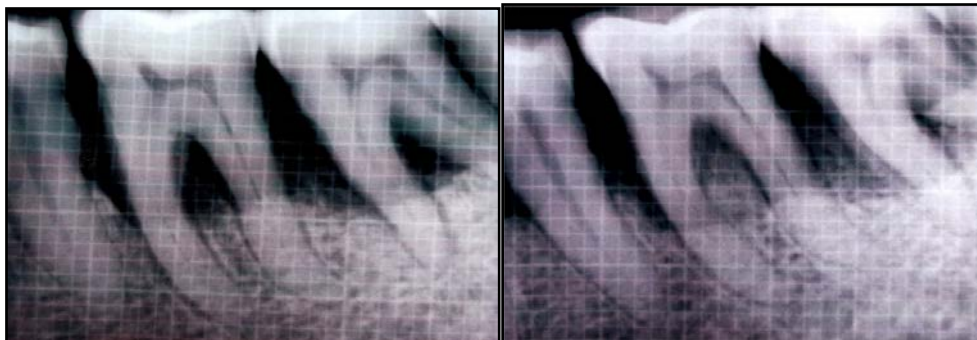
#### **Presurgical procedure-**

Following an initial examination and treatment planning, all the selected patients were given detailed instructions in self performed plaque control measures and were subjected to phase I periodontal therapy. Six weeks after phase I therapy, aperiodontal evaluation was performed to confirm the suitability of the sites for this study. The elected patients were subjected to surgical procedure. The lower/apical

limit of vertical grooves of acrylic guide stent was used as the fixed reference point for the vertical and horizontal probing depths. [4] [figure 1 and 2]. All the sites in both experimental and control groups were subjected to second radiographic assessment. [Figure 3 and 4] Digital intraoral Radiovisiograph were taken for each site after 6 months along with Grid and hard tissue measurements were analysed by using Adobe software.



**Figure 3: Radiovisigraph showing Control site in 46 and 47 furcation area (A) pre operatively, and (B) post operatively**



**Figure 4: Radiovisigraph showing Test site 36 & 37 (Furcation region) (A) pre operatively (B) post operatively**

#### **Surgical management-**

The patient was asked to rinse his/her mouth with 0.2% chlorhexidine gluconate for 1 minute to ensure asepsis and infection control prior to the surgical procedure. The area to be treated was thoroughly anesthetized by regional block using 2% lignocaine hydrochloride (1:80,000).

After adequate anaesthesia, the sulcular incisions were made, to preserve the existing, keratinized gingival tissue as much as possible. The incision extends to minimum of two teeth mesially and one tooth distally to the tooth being treated on both buccal and lingual sides of the operated teeth. [5] A periosteal elevator (P24G) was used to elevate the full thickness mucoperiosteal flap from the bone by moving it mesially, distally and apical until the desired reflection was achieved. Meticulous defect debridement and root planing were carried out. The furcation defect was carefully curetted so

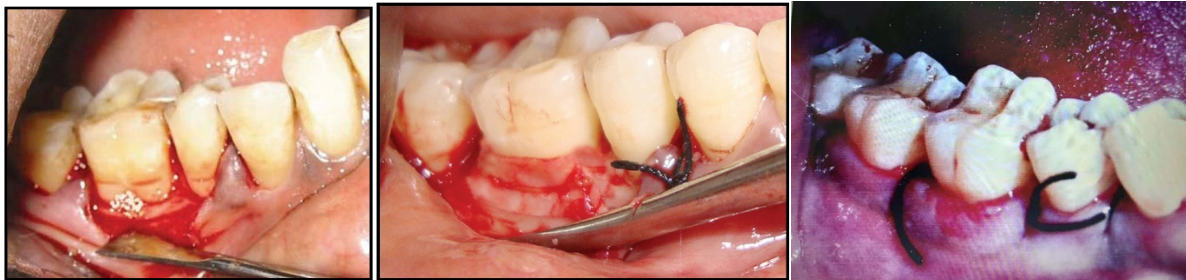
that the entire bone and the root surface adjacent to the teeth can be assessed.

The defect sites in each patient were randomly assigned to the one of the treatment procedures after complete debridement of the defect. The control sites were treated with barrier membrane (Healiguide) and bone graft. The test sites were treated with Platelet Rich Fibrin (PRF) alone by covering the furcation area as a barrier membrane [Figure 5 and 6]. The membrane is carefully placed so that apical border of the GTR membrane extends 3 or 4 mm apical to the margin of the defect and laterally 2 to 3 mm beyond the defect; the occlusal border of the membrane is placed 2 mm apical to the cemento-enamel junctions.

The mucoperiosteal flaps were repositioned and sutured in its original position or slightly coronal to it by interrupted independent sutures such that the flap covers the membrane completely using silk non-absorbable surgical needles

sutures (Ethicon) with 3-0 metric, 20 mm, ½ circle, reverse cutting needle. [6] The surgical area was covered with non-

eugenol periodontal dressing (Coe -pack, G C America Inc, USA).



**Figure 5: Control site in 46 and 47 furcation area bone graft placements with GTR membrane covering and mucoperiosteal flap repositioning with sutures.**



**Figure 6: Test site in 36 and 37 furcation area PRF, used as the membrane to cover the furcation defect area and mucoperiosteal flap repositioning with sutures.**

All patients were prescribed with systemic Antibiotics; Amoxicillin 500 mg+ Clavulanic acid 125 mg (Moxikind-CV) tablets t.d.s for 5 days and analgesics: Diclofenac 50 mg + Paracetamol 325mg (Diclomed) tablet twice daily for 5 days. The patients were instructed to avoid mechanical tooth brushing and to chew carefully, from the operated site, for 1 weeks. [7] Mouth rinse 10 ml of 0.2 % chlorhexidine gluconate solution was

prescribed twice daily for 4 weeks to maintain oral hygiene in the operated sites. On the second day following surgery, patients were recalled and asked regarding any swelling, discomfort, pain and /or sensitivity, after 1 week's periodontal dressing and sutures were removed. Patients were reinstructed for proper oral hygiene measures postoperatively, and examined weekly upto 1 month after surgery, and again, at 3 and 6 months.



**Figure 7: Control site 46 b) post operative -PPD (from gingival margin to the base of the pocket), VCAL (from fixed reference point to the base of pocket) and GML (from fixed reference point to the gingival marginal level).**



**Figure 8: Test site 37 b) post operative -PPD (from gingival margin to the base of the pocket), VCAL (from fixed reference point to the base of the pocket ) and GML (from fixed reference point to the gingival margin)**

#### Data analysis-

Pairwise comparison within the groups were done by applying student 't'test. Comparison were also drawn for the changed mean plaque and gingival scores, Relative clinical attachment levels (horizontal vertical), gingival marginal level and probing pocket depth between the control (GTR+ BG) and the test (PRF) groups by applying the independent student 't'test.

#### Results

All the 20 subjects completed the study. No adverse event of any kind occurred during the course of the study. Membrane exposure was not seen in any of the cases.

The mean change in the gingival scores for both the test and the control group were  $1.20 \pm 0.42$  mm, respectively, at the end of six months, with a difference of 0.05 which was statistically significant. The mean change in the plaque scores for test and control groups were  $1.30 \pm 0.48$  mm which was not statistically significant and

$1.20 \pm 0.42$  mm which was statistically significant.

The relative horizontal clinical horizontal clinical attachment level of test group was  $1.80 \pm 3.35$  mm and control group was  $1.60 \pm 1.5$  mm with a mean difference of  $0.2 \pm 0.38$  mm with p-value being 0.79 which is not significant.

The relative vertical clinical attachment level of the test group was  $1.30 \pm 0.06$  mm and control group were  $1.70 \pm 0.67$  mm with a mean difference of  $0.4 \pm 0.30$  mm with the p-value being 0.13 which is not significant.

The gingival marginal level of the test group was  $0.40 \pm 0.51$  mm and control group was  $0.20 \pm 1.31$  mm with a mean difference of  $0.2 \pm 0.31$  mm with a p-value being 0.09 which is not significant.

The bone density of the test group was  $10.06 \pm 47.08$  mm and control group were  $17.46 \pm 11.87$  mm with a mean difference of  $7.4 \pm 5.09$  mm with a p-value of 0.06 which is not significant.

**Table 1: SBI Index score, Chi square, Mean  $\pm$ SD and Df of Test and Control group in baseline and six months**

Group	Visit	SBI Index score			Chi square	Mean $\pm$ sd	Df	P value
		0-0.5	0.6-1.0	1.1-1.5				
Control group	Baseline	0 (0.0)	4 (40.0)	6 (60.0)	0.400	$2.60 \pm 0.51$	1	0.52
	6 months	8(80.0)	2(20.0)	0 (0.0)	3.600	$1.20 \pm 0.42$	1	0.05
Test group	Baseline	0(0.0)	7(70.0)	3(30.0)	1.600	$2.30 \pm 0.48$	1	0.20
	6 months	8(80.0)	2(20.0)	0(0.0)	3.600	$1.20 \pm 0.42$	1	0.05

**Table 2: Plaque Index score, Chi square, Mean  $\pm$ SD and Df of Test and Control group in baseline and six months**

Group	Visit	Plaque Index score			Chi square	Mean $\pm$ SD	Df	P-value
		0-0.5	0.6-1.0	1.1-1.5				
Control group	Baseline	0(0.0)	6	4	0.400	2.40 $\pm$ 0.51	1	0.52
	6 months	8(80.0)	2	0	3.600	1.20 $\pm$ 0.42	1	0.05
Test group	Baseline	0(0.00)	6	4	0.400	2.40 $\pm$ 0.51	1	0.52
	6 months	7(70.0)	3	0	1.600	1.30 $\pm$ 0.48	1	0.20

**Table 3: Relative vertical clinical attachment level of Test and Control group**

RVCAL (mm)	Visit	Mean $\pm$ sd	T value	P value	Mean difference $\pm$ SD	T value	P-value
Control group	Baseline	9.19 $\pm$ 1.59	18.04	0.00	1.70 $\pm$ 0.67	7.96	0.00
	6 months	7.40 $\pm$ 1.34	17.35	0.00			
Test group	Baseline	10.80 $\pm$ 2.09	16.28	0.00	1.30 $\pm$ 0.67	6.09	0.00
	6 months	9.50 $\pm$ 2.06	14.52	0.00			

**Table 4: Relative Horizontal clinical attachment level of Test and Control group**

RHCAL (mm)	Visit	Mean $\pm$ sd	T value	P value	Mean difference $\pm$ SD	T value	P-value
Control group	Baseline	8.90 $\pm$ 1.85	15.18	0.00	1.60 $\pm$ 1.5	3.36	0.08
	6 months	7.30 $\pm$ 2.40	9.59	0.00			
Test group	Baseline	10.50 $\pm$ 2.27	14.60	0.00	1.80 $\pm$ 3.35	1.67	0.12
	6 months	8.70 $\pm$ 1.94	14.13	0.00			

**Table 5: gingival margin level in Test and Control group**

GML (mm)	Visit	Mean $\pm$ sd	T value	P value	Mean difference $\pm$ SD	T value	P-value
Control group	Baseline	3.50 $\pm$ 1.61	7.42	0.00	0.20 $\pm$ 1.31	0.48	0.64
	6 months	3.70 $\pm$ 1.15	10.09	0.00			
Test group	Baseline	3.20 $\pm$ 1.13	8.91	0.00	0.40 $\pm$ 0.51	1.30	0.22
	6 months	3.60 $\pm$ 0.84	13.50	0.00			

**Table 6: Bone density in Test and Control group**

Bone density (mm)	Visit	Mean $\pm$ sd	T value	P value	Mean difference $\pm$ SD	T value	P-value
Control group	Baseline	96.24 $\pm$ 35.38	8.60	0.00	17.46 $\pm$ 11.87	4.63	0.00
	6 months	113.74 $\pm$ 30.30	11.86	0.00			

Test group	Baseline	90.80±25.84	11.11	0.00	10.06±47.08	0.66	0.52
	6 months	100.86±47.16	10.11	0.00			

**Table 7: Probing pocket depth of Test group and Control group in baseline and six months**

Probing pocket depth	T	Sig.	Mean Difference
Test group (baseline)	24.832	.000	5.30±0.67
Test group (6 months)	10.474	.000	2.90±0.87
Control group (baseline)	33.000	.000	5.50±0.52
Control group (6 months)	15.922	.000	2.60±0.51

**Table 8: Probing pocket depth of Test group and Control group**

Probing pocket depth (mm)	Visit	Mean difference± SD	T value	P- value
Control group	Baseline	2.90±0.99	9.22	0.04
	6 months			
Test group	Baseline	2.40±0.96	7.66	0.49
	6 months			

**Table 9: Plaque Index (PI), Sulcus bleeding index (SBI), Probing depth from the gingival margin, Relative vertical clinical attachment level (RVCAL), Relative Horizontal clinical attachment level (RHCAL) along with gingival margin level (GML) and BD of Test and Control group, mean difference, T value and P value.**

Parameters	Test Group	Control Group	Mean Difference	T-Value	P-Value	
PD	2.40±0.96	2.90±0.99	0.5±0.31	4.00	0.64	Not Significant
RHCAL	1.80±3.35	1.60±1.5	0.2±0.38	0.20	0.79	Not Significant
RVCAL	1.30±0.06	1.70±0.67	0.4±0.30	2.4	0.13	Not Significant
GML	0.40±0.51	0.20±1.31	0.2±0.31	0.48	0.09	Not Significant
BD	10.06±47.08	17.46±11.87	7.4±5.09	1.56	0.06	Not Significant

## Discussion

Furcation involvement complicates the treatment methodology due to its bacterial retentive nature and anatomical factors which prevents visualization and access for treatment.[8] According to Caranza and Newman, furcation involvement

microscopically is simply a phase in the rootward extension of the periodontal pocket. In its early stages, there is a widening of the periodontal space, with cellular and fluid inflammatory exudation, followed by epithelial proliferation into the



furcation area from the adjoining periodontal pocket.

Caranza noted that trauma from occlusion is particularly suspected when the furcation involvement demonstrated crater like or angular deformities in the bone and especially when the bone destruction is localized to one of the roots. Regenerative modalities for the treatment for grade II furcation defect include use of osseous grafting with osseous coagulum, autogenous intraoral bone, iliac crest bone, freeze dried bone with autogenous bone and hydroxyapatite, GTR membranes/PRP alone, GTR/PRF/PRP in conjunction with bone grafting materials. [9]

Treatment of furcation involvement depends largely on the extent of lesion. Shallow horizontal involvement without significant vertical bone loss usually responds favorably to localize flap procedures with odontoplasty and osteoplasty. [9]

Collagen material also possess additional advantages like hemostasis and chemotaxis for periodontal ligament fibroblasts, reduces immunogenicity, easy manipulation and ability to augment tissue thickness, Hence, collagen membranes are ideal for resorbable GTR membranes. [10] Healguide is commercially available resorbable membrane with type I collagen as major component. Additional charge notifications and slight calcification makes this membrane unique and different from other membranes known to facilitate fibrogenesis over osteoinduction. Also, its pore size is lesser than the penetrable size of an epithelial cell migrating from gingival /periodontal flap during the initial phases of healing.

PRF is an autogenous platelet concentrate which consist of a fibrin matrix polymerized in a tetramolecular structure, the incorporation of platelets, leukocytes and cytokines; and circulating stem cells. In addition, PRF slows down the blood activation process, which could

induce an increased leukocyte degranulation and release of cytokines (proinflammatory mediators) such as interleukin (IL)-1beta, IL -6 and tumor necrosis factor alpha, and anti-inflammatory cytokines such as IL-4. [11] Thus, in order to evaluate regeneration in grade II furcation defects using the above-mentioned modality the following parameters were recorded at baseline and 6 months - *Plaque index, bleeding index, Probing depth, Relative vertical attachment level, Relative horizontal attachment level, Gingival margin level and Radiographic bone density.*

Belal et al [12] also observed a mean reduction of 2.43 mm with control group & 2.5 mm with test group. However, Sanatana et al reported a higher PPD reduction (3.65 -0.6 mm) by using a composite graft composed of bioabsorbable hydroxyapatite mixed with tetracycline hydrochloride and PTFE barrier membrane.

Quick handling is the only way to obtain a clinically usable PRF. [13] The technique of PRF preparation was followed carefully and it was prepared immediate before placement into the defect membrane were prepared for each test group site to maintain the basic rules of tissue engineering as prepared by Corso et al. [14]

Moreover, the fibrin matrix itself shows mechanical adhesive properties and biologic functions like fibrin glues: it maintains the flap in a high and stable position, enhances neo angiogenesis, reduces necrosis and shrinkage of the flap, and guarantees maximal root coverage. [15] Because it is a simplified, easy, fast and cost-effective preparation without use of any anticoagulant, along with functional, intact platelet in fibrin matrix and sustained release of growth factors, PRF is considered the leader in fibrin technology. [16]

The current study was also demonstrated decrease in the gingival index scores from baseline to 6 months & this decrease was statistically significant. This demonstrates that both PRF & HEALIGUIDE are biocompatible material and does not stimulate any inflammatory reaction in the soft and hard tissues.

### Conclusion

Both treatment modalities demonstrated a significant improvement in the probing depth, vertical and horizontal relative attachment level as well as radiographic bone density at 6 months surgery. There was no significant difference in the result obtained from the use of both the treatment modalities. Hence further long-term studies are requiring substantiating the efficacy of cost effective PRF in the treatment of mandibular grade II furcation defects over other GTR membranes which are presently being used.

### References

1. Mrinalini Ag, Bhatnagar, Deepa D. Management of grade II furcation defect in mandibular molar with alloplastic bone graft and bioresorbable guided tissue regeneration membrane. *J Periodontol* 2016; 1:96-99.
2. Dr. Tharangini, Dr Mohan valiathan. Plasma rich fibrin in treatment of grade 2 furcation management – A systematic review. *J Clin Periodontol*. 2020; Vol 07, Iss 03.
3. Dr. A R Pradeep, Dr. Nishanth S. Rao et al. Comparative Evaluation of Autologous Platelet rich fibrin and platelet rich plasma in the treatment of three wall intrabony defects in Chronic Periodontitis: A Randomized Controlled Clinical Trial. *J Periodontol*. 2012;83(12):1499-507.
4. L. Benamghar, J. Penaud et al. Comparison of gingival index and sulcus bleeding index as indicators of periodontal status. 1982; 60(1): 147–151
5. Chung KM, Salkin LM, Stein MD et al. Clinical Evaluation of a Biodegradable Collagen Membrane in Guided Tissue Regeneration. *J Periodontol*; 1990; 61:732-736
6. Kobayashi M, Kawase T et al. A proposed protocol for the standardized preparation of PRF membranes for clinical use. *Biologicals* .2012;40: 323-329
7. Gerald M Bowers et al. Factors influencing the Outcome of Regenerative Therapy in Mandibular Class II furcations. Part I *J Periodontol* 2003;74: 1255-1268
8. Gerald M Bowers et al. Factors influencing the Outcome of Regenerative Therapy in Mandibular Class II furcations. Part I *J Periodontol* 2003;74: 1255-1268
9. Waerhaug, Jens. The Infrabony Pocket and its Relationship to Trauma from Occlusion and Subgingival Plaque. *J Periodontol* .1979 ;50: 355–365
10. Pintippa Bunyaratvej and Hom -Lay wang. Collagen Membranes: A Review *J Periodontol* 2001;72;215-229
11. Joseph Choukroun MD. Platelet -rich fibrin (PRF): A second generation platelet concentrate. Part IV: Clinical effect on issue healing. *Ora surg Oral Med Ora Radiol Pathol Oral Endod* 2006; 101:56-60
12. Machtei E. Outcome Variables for the Study of Periodontal Regeneration. *Ann of Periodontol* .1997; 2: 229–239
13. Marcelo Camelo, Maec L. Nevins, et al. Periodontal regeneration in human class II furcations using Purified recombinant human platelet derived growth factor -BB with bone allograft, *Int J Periodontics Restorative Dent* 2006; 23:213-225
14. Meyle J. Periodontics: Current concepts and emerging trends. *Int J Dent Hyg* 2006; 43–49
15. David M. Dohan, et al. Platelet -rich fibrin (PRF): A second -generation platelet concentrate. Part I: Technological concepts and evolution

Oral Surg Oral Med Oral Pathol Radiol  
Endod 2006; 101:37-44.  
16. Hays, P. Evidence Basis for  
Pharmacogenetic Testing in

Psychiatry. Journal of Medical  
Research and Health Sciences,  
2022;5(3), 1838–1859.