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Comparative Evaluation of Platelet Rich Fibrin Membrane vs. Collagen Membrane in Combination with Hydroxy-Apatite (HA) for the Treatment of Mandibular Molar Furcation Defects – A Randomized Clinical Control Trial

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Abstract

Aim: A clinical trial to compare the relative efficacy of hydroxyapatite in combination with either PRF (Platelet rich fibrin) or collagen membrane in treatment of grade II furcation defects of mandibular molars. **Methodology:** The sample consisted of 35 Grade II mandibular molar furcation defects planed for open flap debridement (OFD) (Group 1, n=12), Platelet-rich fibrin membrane + Hydroxyapatite (HA) with OFD (Group 2, n=12), and Collagen membrane (CM) + HA with OFD (Group 3, n=11). These were recorded at baseline and 9 months postoperatively for plaque index (PI), gingival index (GI), probing depth (PPD), relative vertical and relative horizontal clinical attachment levels (RVCAL and RHCAL), and intrabony defect depth (IBD). **Results:** PI and GI scores decreased significantly in all three groups at 9 months postoperatively as compared to baseline scores. After 9 months of follow up visits mean PD reduction was greater in Group 2 (3.66 ± 0.98 mm) and Group 3 (3.45 ± 1.5 mm) than Group 1 (2.08 ± 0.90 mm), and mean RVCAL and RHCAL gain were greater in Group 2 (3.25 ± 0.75 mm and 3.92 ± 0.29 mm, respectively) and Group 3 (3.09 ± 0.70 mm and 3.89 ± 0.54 mm) compared with Group 1 (1.33 ± 0.77 mm and 2.25 ± 0.62 mm). A significant reduction in IBD depth was found in Groups 2 (2.16*0.38 mm) and 3 (1.58*0.35 mm) than in Group 1 (0.70*0.15 mm). The differences in PI, GI, PPD, RVCAL, and RHCAL between Groups 2 and 3 at baseline and 9 months were not statistically significant. There was a statistically significant difference in the depth reduction of IBD between Groups 2 and 3 at nine months. **Conclusion:** Within the scope of this study, it can be stated that the use of HA in conjunction with PRF or CM in the treatment of grade II mandibular furcation abnormalities resulted in a considerable reduction in GI PI, PPD, RVCAL, RHCAL, and IBD depth on follow up visit at 9 months after surgery. However, the combination of HA with PRF membrane exhibited significant improvement in radiographic parameters in comparison to the combination of HA with Collagen membrane.

Keywords: Collagen Membrane, Mandibular Molar Furcation, Open Flap Debridement, Study

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1. Introduction

The anatomic site of a multirooted tooth where the roots diverge is termed furcation. Its result of, pathologic resorption of the supporting alveolar bone within a furcation", which is derived from the progression of periodontitis.[1-3] One of the most prevalent dentoalveolar consequences of periodontal disease is molar tooth furcation involvement. A complete grasp of tooth anatomy, causative variables, and the biologic basis for treatment modalities is required to apply a specific treatment technique for furcation involvement. Systemic factors like diabetes and smoking, as well as local factors like cervical enamel projections, furcation entry width, furcation and root concavities etc. contribute to furcation involvement[1,4,5].

To treat furcation defects, a variety of treatments have been proposed, ranging from conservative methods like curettage to surgical therapeutic approaches like gingivectomy, root amputation, hemi-section, or tunneling to enhanced alternative treatments like bone grafting, Guided Tissue Regenerative (GTR) procedures, or combination therapy (GBR)[1,4-6].

In grade II and III furcation defects, it has been shown that combining either a resorbable or non-resorbable membrane with bone grafts for mandibular first and second molars enhanced defect fill, periodontal probing depth decrease, and clinical attachment gain compared to a barrier alone[4].

Clinical studies have been conducted for treatment of intra-bony defects on a variety of bone graft materials, including intraoral and extraoral autografts, allografts, xenografts, and synthetic/semi-synthetic materials (alloplastic) like Hydroxyl-Apatite (HA), tricalcium phosphate, and bioactive glass, etc[7].

HA is a commonly used alloplastic bone graft material found in two forms: non-porous non-resorbable and porous resorbable. Resorbability is a property by which graft material is to be replaced by host bone. Using HA to fill periodontal intra-bony defects produced a clinically satisfactory result. Porous HA bone grafts have been shown to have good bone conducting capabilities, allowing osteogenic cells to grow from existing bone surfaces into surrounding bone material. Because HA contains no organic substances, it does not cause an allergic response and is well tolerated in clinical trials[8,9].

For periodontal regeneration, different types of membranes are employed to cover periodontal deficiencies. A membrane should be biocompatible, allow for cell exclusion, maintain space, allow for tissue integration, and be simple to utilise[10]. In nature, membranes can be non-absorbable or absorbable. Non-absorbable membranes necessitate a second surgery to remove hence they are used less commonly nowadays. Absorbable membranes don't need to be removed, which means less pain for the patient and fewer surgical complications. Natural materials such as collagen, pericardium, dura mater, periosteum, PRP or PRF membranes, as well as synthetic materials such as polyglycolic acid, polylactic acid or polydioxanone, can be used to create absorbable membranes[1,11].

Choukroun *et al.* (2001)[12] described Platelet-Rich Fibrin (PRF), a second generation[13] platelet concentrate that allows one to extract fibrin membranes loaded with platelets and growth factors. PRF resembles a fibrin network and promotes cell migration and proliferation, resulting in cicatrization. This peculiar structure could be used to transport cells necessary for tissue repair. The PRF membrane could also be used as a resorbable membrane for tissue regeneration (GTR)[14]. Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF), and Transforming Growth Factor (TGF-b) are among the growth factors generated by PRF. It stimulates the release of cytokines such as IL-1, IL-4, IL-6, and TNF- alpha[15,16]. Recent research has shown that the PRF membrane has a considerable slow continuous release of essential growth factors for at least one week and up to 28 days, implying that the membrane stimulates its environment for an extended period of time during wound healing[17].

Collagen is the body's most abundant protein and the primary component of periodontal connective tissue. It makes up about 30% of total protein in the human body and serves as the extracellular framework for all multicellular animals. It's a biodegradable membrane[18].

Periodontal therapy's ultimate goal is to repair periodontal tissues that have been destroyed owing to periodontitis[19]. Hence, combining either PRF membrane or collagen membrane with HA for periodontal regeneration therapy is an intriguing and potentially helpful technique for treating periodontal osseous abnormalities.

To the best of our knowledge, nine-month follow-up assessment of a combination of HA with PRF or collagen membrane with HA in the management of intrabony osseous defects or furcation involvement defects has yet to be done. Thus the purpose of this study was to compare the efficacy of autologous PRF membrane with HA and collagen membrane with HA bone graft with Open Flap

Debridement (OFD) to OFD alone in the treatment of degree II furcation defects in the mandible.

2. Material and Methods

2.1 Patient Selection

A total of 36 surgical sites in 20 systemically healthy individuals (11 males, 9 females) were enrolled in this randomised experiment from the outpatient branch of the RUHS College of Dental Sciences in Jaipur, India. Patients were tracked for up to 9 months in this single-center, parallel-arm design research. The research took place between December 2016 and November 2018. The Institutional Ethical Committee approved the study procedure, which followed the Helsinki Declaration[20]. The method was explained to participants in their native language, and written informed consent was obtained.

Patients with Glickman's Grade II furcation abnormalities buccally in vital, asymptomatic mandibular first and second teeth met the inclusion criteria. On an Intra-Oral Periapical Radiograph (IOPA), radiolucent furcation region with vertical Probing Depth (PD) of 5mm and horizontal Probing Depth (PD) of 3mm. The study excluded individuals who had received periodontal therapy, smokers, pregnant and breastfeeding women, teeth with Grade II mobility, and patients who were using drugs.

2.2 Sample Size Estimation

The minimum required sample size per group was n=10 sites, assuming 80 percent power, a 5% significance level with a 95 percent confidence interval, and a standard 0.50. A minimum sample size of 12 sites per group was recommended, assuming a 20% loss rate during follow-up[21].

2.3 Randomization

Test sites received HA bone graft material with PRF membrane in one group and HA bone graft material with collagen membrane in another group, while control sites underwent OFD alone. Samples were randomised into either of the groups using a simple coin toss method.

2.4 Stent Preparation

The stent was produced using the sprinkle method and cold cure acrylic. The stent was around 2-3 mm thick. The UNC-15 probe was guided into the vertical grooves on the buccal and lingual sides of the stent using a straight fissure bur no. 556 and an air-rotor hand piece. The stent was cut to 1/3 on the occlusal/incisal surfaces of the teeth, and the measurements were taken using the UNC 15 periodontal probe, which was inserted into the stent's groove[22].

2.5 Clinical Parameters

1. Plaque Index (PI) (Silness and Loe 1964)
2. Gingival Index (GI) (Loe and Silness 1963)
3. Probing pocket depth (PPD).
4. Relative Vertical Clinical Attachment Level (RVCAL) was calculated by measuring the distance from the Reference Point (RP) (fabricated occlusal stent) to the Base Of Pocket (BOP) and subtracting by the distance from the reference point to the Cement-Enamel Junction (CEJ).
   CAL = (RP-BOP) – (RP-CEJ).
5. Relative horizontal clinical attachment level (RHCAL) at furcation defect was measured from a fixed reference point in to furcation using colour coded graduated Nabers's probe marked at 3 mm intervals.

2.6 Radiographic Parameters

CBCT images were taken at base line and 9 months postoperatively. The radiographic parameters which were recorded are:

1. Intra Bony Defect (IBD) depth
   The distance between the furcation fornix and the base of the defect was used to calculate the size of the bone defect.
2. Intra Bony Defect Reduction
   Defect depth at baseline – defect depth post operatively.

2.7 Pre-Surgical Therapy

Before the procedure, each patient was given oral hygiene instructions and precautions. A full mouth Scaling and Root Planning (SRP) was performed using ultrasonic and manual instruments and a local anaesthetic solution. These patients were randomly assigned to one of three groups. Patients were masked for specific group and treatment allocation.

2.8 PRF Membrane Preparation Protocol

Platelet rich fibrin a second generation platelet concentrate was prepared according to the protocol developed by

Choukroun *et al.* (2001)[23]. It was prepared in the form of a membrane by squeezing out the fluids present in the fibrin clot.

2.9 Collagen Membrane

Healguide (Advanced Biotech Products, Chennai) collagen membrane was used in the stud.

2.10 Surgical Protocol

A full thickness mucoperiosteal flap was lifted, and area-specific Gracey curettes were used to perform thorough root debridement and curettage. Any embedded calculus in the furcation defect was meticulously inspected. A sterile normal saline solution was used to irrigate the operative region. Prior to grafting, non-resorbable 3-0 black braided silk was used for pre-suturing. There was no attempt at osseous recon touring. These patients were randomly assigned to one of three groups.

***Group 1* (OFD):** Only conventional surgery was done, with thorough debridement of furcation area.

***Group 2* (OFD+HA+PRF membrane):** After thorough Open flap debridement, HA was placed into the furcation defect. PRF membrane was trimmed and placed to cover furcation defect.

***Group 3* (OFD+HA+CM):** After thorough Open flap debridement. HA was placed into the furcation defect. Collagen membrane was trimmed and placed to cover furcation defect.

3-0 silk sutures, interrupted or sling sutures were used to reposition the whole thickness flap. The surgical region was covered with periodontal dressing to protect it. Each patient was given specific post-operative instructions.

2.11 Post-Operative Care

Antibiotics and analgesics were given. A two-week course of Chlorhexidine mouthwash (0.2 percent) was also suggested. One week following surgery, the suture and periodontal dressing were removed.

2.12 Post-Surgical Measurements

At 9 months after surgery, clinical data were assessed. For soft tissue measures, acrylic stents were applied which were previously used. Another CBCT of the same surgical site was taken for hard tissue assessment (bony defect measurement), and the measurement was re-evaluated after 9 months.

2.13 Statistical Analysis

All the data were calculated and statistically analysed with SPSS 20.0 computer software package for windows (SPSS INC. Chicago, IL, US). The data is expressed as mean ± SD and tested for variance. Inter group comparison among the groups for PI, GI, PPD, RVCAL, RHCAL and IBD depth reduction were statistically analysed with one-way ANOVA followed by Tukey's multiple test as a post hoc test. Paired t test was used to compare the data within each group. Differences in means were considered significant at $p<0.05$.

3. Results

The present study was undertaken to evaluate the comparative efficacy of HA with either Platelet Rich Fibrin (PRF) Membrane or Collagen Membrane (CM) in the treatment of grade II Mandibular molars furcation defects. A total of 36 grade II molar defects in 20 patients (11 Males, 9 Females) of mean age of 41.3± 6.8 years were treated in 3 groups. These patients were divided into three groups randomly. One patient dropped during follow up and scheduled maintenance visits, so total 19 patients (10 Males, 9 Females) with 35 surgical sites completed study (Table 1 & Figure 1).

Clinical and radiographic parameters evaluation were performed at base line (pre-operative) and post operatively 9th months, all regenerative materials (PRF membrane, CM and HA) were found to be well tolerated and surgical sites healed without any complications. Baseline analysis did not display any significant difference among all the three groups for any of clinical and radio graphical variable (parameters) assessed, suggesting that final differences between treatment groups were not influenced by initial defect characteristics, thus allowing post treatment results to be compared. Intra group comparison in all groups between base line and of 9th month showed significant difference for all clinical and radio graphical parameters (Table 2).

The mean difference of the scores of PI and GI between baseline and of 9th months was statistically significant. Inter group comparison among the three groups at baseline and at 9th month's post-intervention durations did not depict any significant difference. Groups 2 & 3 presented with significant greater reduction in PPD (3.66±0.98mm, 3.45±1.50mm respectively) than Group 1 (2.08±0.90mm)

Table 1. Demographic details of study population

Parameters	Group 1	Group 2	Group 3	p value	
No of Patients	7	6	6	Group 1 vs. 2	0.586 (NS)
				Group 1 vs. 3	0.586 (NS)
				Group 2 vs. 3	0.947 (NS)
No of Furcation defects	12	12	11	Group 1 vs. 2	0.989 (NS)
				Group 1 vs. 3	0.532 (NS)
				Group 2 vs. 3	0.532 (NS)
Age (Mean Years)	41.7±6.90	39.8±6.3	42.5±7.1	Group 1 vs. 2	0.598 (NS)
				Group 1 vs. 3	0.898 (S)
				Group 2 vs. 3	0.524 (NS)

†p value< 0.05 – significant (S), NS- non significant

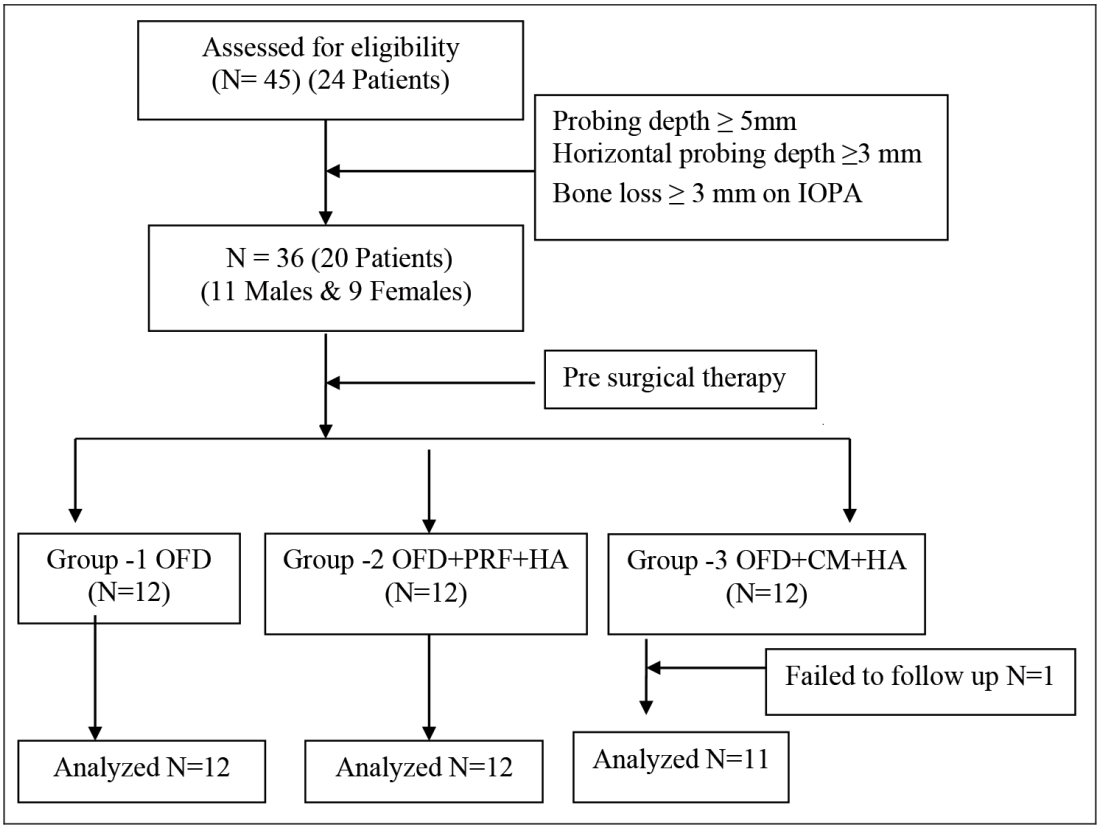


Figure 1. Study flow chart.

at the end of 9 month post operatively. Inter group comparison among the group 2 vs. 3, 9th months post operatively did not depict any significant difference.

Group 2 (3.25±0.75mm) and 3 (3.09±0.70 mm) are presented with significant greater gain in RVCAL than Group 1 (1.33±0.77mm) at the end of 9th month post operatively. Inter group comparison among the group 2 vs. 3 at the end of 9th month post operatively did not depict any significant difference. Both Group 2 & 3 presented with significant greater gain in RHCAL than Group 1 at the end of 9 months post operatively (2.25±0.62mm, 3.92±0.29mm, 3.89±0.54mm respectively). Inter group

comparison among the group 2 *vs.* 3 at the end of 9th month post operatively did not depict any significant difference. The mean reduction of IBD depth between base line and 9 months were 0.70±0.15mm, 2.16±0.38mm, 1.58±0.35mm respectively in Group 1, Group 2 and Group 3, which was statistically significant (Table 3).

Table 2. Clinical and radiographic parameters in all 3 groups at baseline and 9 months

Parameters	Visits	Group 1		Group 2		Group 3	
		Mean ± S.D.	p - value	Mean ± S.D.	p – value	Mean ± S.D.	
PI	Baseline	2.38 ± 0.34	0.00*	2.46 ± 0.42	0.00*	2.59 ± 0.28	0.00*
	9 Months	0.60±0.23		0.54 ± 0.21		0.61 ± 0.17	
GI	Baseline	2.51±0.26	0.00*	2.53±0.20	0.00*	2.48±0.20	0.00*
	9 Months	0.58 ± 0.27		0.58 ± 0.27		0.57 ± 0.20	
PPD	Baseline	8.41±0.90	0.00*	8.33±0.78	0.00*	8.45±1.03	0.00*
	9 Months	6.33±0.78		4.66±0.89		5.01±1.26	
RVCAL	Baseline	9.66±0.98	0.034*	9.58±0.90	0.00*	9.72±0.90	0.00*
	9 Months	8.33±0.65		6.34±0.15		6.63±1.02	
RHCAL	Baseline	8.20±0.93	0.00*	8.60±0.79	0.00*		0.00*
	9 Months	5.91±0.66		4.66±0.65			
IBD	Baseline	4.62±0.40	0.00*	4.72±0.29	0.00*	4.53±0.37	0.00*
	9 Months	3.93±0.35		2.56±0.52		2.99±0.36	

†p value< 0.05 – significant (S), NS- non significant.

Table 3. Mean changes in the clinical and radiographic parameters over 9 month's period inter groups

Para-meters	Group 1	Group 2	Group 3	p value	
Mean PI change	1.77 ± 0.47	1.92 ± 0.46	1.98 ± 0.34	Group 1 vs. 2	0.686 (NS)
				Group 1 vs. 3	0.490 (NS)
				Group 2 vs. 3	0.939 (NS)
Mean GI changes	1.91±0.39	1.93±0.38	1.90±0.32	Group 1 vs. 2	0.989 (NS)
				Group 1 vs. 3	0.998 (NS)
				Group 2 vs. 3	0.981 (NS)
Mean PPD change (mm)	2.08±0.90	3.66±0.98	3.45±1.5	Group 1 vs. 2	0.005† (S)
				Group 1 vs. 3	0.020† (S)
				Group 2 vs. 3	0.898(NS)
Mean RVCAL gain (mm)	1.33±0.77	3.25±0.75	3.09±0.70	Group 1 vs. 2	0.000† (S)
				Group 1 vs. 3	0.000† (S)
				Group 2 vs. 3	0.867(NS)
Mean RHCAL gain (mm)	2.25±0.62	3.92±0.29	3.89±0.54	Group 1 vs. 2	0.000† (S)
				Group 1 vs. 3	0.000† (S)
				Group 2 vs. 3	0.999(NS)
Mean IBD depth reduction (mm)	0.70±0.15	2.16±0.38	1.58±0.35	Group 1 vs. 2	0.000† (S)
				Group 1 vs. 3	0.000† (S)
				Group 2 vs. 3	0.044 (S)

†p value< 0.05 – Significant (S), NS- Non Significant.

4. Discussion

This study aims to compare the clinical efficacy of autologous PRF as a membrane with HA and comparing it with Collagen membrane with HA in the treatment of Grade II mandibular molar furcation involvement in human subjects.

Because changes in root shape among teeth can influence treatment outcome, the treatment sites were limited to mandibular molars only[24]. Clinical and radiographic criteria that give surrogate proof of periodontal regeneration were used in the study due to ethical and patient concerns[25]. At baseline and 9 months, clinical and radiographic data were examined.

Because of its high resolution and three-dimensional capabilities to analyse buccal and lingual surfaces, CBCT analysis was employed in this study for radiographic assessment of bone defect fill. This makes it a particularly reliable tool for diagnosing incipient furcation involvement[26-28].

In the current study, all three groups experienced a significant reduction in PI and GI scores after 9 months when compared to baseline levels. The mean value of PI and GI scores at baseline and 9 months post-operatively did not demonstrate any statistical difference between the three groups. The current study's findings are consistent with previous research[29-31].

This decrease in GI and PI scores reported in this study could be due to greater patient compliance with regular oral hygiene instructions, allowing for enhanced plaque control efficiency[32].

The treatment in all the groups was well tolerated by the host tissue and HA used with either PRF membrane or CM in the present study were biocompatible clinical materials, and these did not enhance plaque accumulation, gingival inflammation or any kind of adverse tissue reaction. These observations are in harmony with the earlier studies[33,34].

The results of this study showed that combining HA with either a PRF membrane or a Collagen membrane was significantly effective in lowering PPD and increasing RVCAL and RHCAL at 9 months post-surgery when compared to OFD alone. These findings support earlier research that shows that combination regenerative therapy produces much better effects than OFD alone[35]. The intergroup comparison of PPD reduction between Groups 2 and 3 at 9 months follow-up visits was insignificant in the current study, which is consistent with previous research[30,31].

The 9-month RHCAL intergroup comparison results are similar with Asimuddin *et al.*[30] However, RVCAL's 9-month data contradict this, reporting a larger increase in the collagen membrane group compared to the PRF membrane group. This discrepancy could be explained by the fact that they employed collagen membrane in combination with allograft, whereas PRF membrane was used alone without any bone graft combination to cure the furcation defect.

Results of present study demonstrated a significant reduction in the IBD at 9 months post operatively in all the three groups when compared with base line data.

The results observed in Group 1 & 2 are parallel with the findings of Pradeep *et al*[29]. The results observed in Group 3 show similar trend with Asimuddin *et al*[30].

On intergroup comparison, both groups 2 and 3 showed a statistically significant reduction in IBD when compared to group 1, implying that the combination of GTR membrane and bone graft is preferable to open flap debridement in the treatment of furcation defects[35].

Furthermore, when comparing the results of IBD decrease in the PRF membrane and CM groups, the PRF membrane group had a substantially greater (p value < 0.04) result than the CM group. This is consistent with the findings of Asimuddin *et al.*[30], who found a highly significant difference in IBD bone fill between the PRF membrane and CM groups. The current study's findings clearly show that PRF membrane + HA combination therapy is superior to CM+HA combination therapy.

The mechanism underlying the PRF membrane group's much improved bone regeneration compared to the collagen membrane group is unknown.

PRF membranes, according to Simonpieri *et al.*[36], are particularly beneficial for periosteum repair and maturation. PRF appears to be superior than collagen as a scaffold for human periosteal cell proliferation, according to Gassling *et al.*[37]. PRF has an inherent osteoconductive and/or osteoinductive characteristic that is favourable to bone regeneration[38].

According to Chang *et al.*[17], PRF increases phosphorylation of Extracellular signal-Regulated protein Kinase (p-ERK) and Osteo-Prote-Gerin (OPG) in periodontal ligament fibroblasts, as well as elevation of Alka-Line Phosphatase (ALP) activity, which leads to osteoblast proliferation. In a similar study, Wu *et al.*[15]

found that PRF can improve osteoblast attachment and proliferation while also up-regulating collagen associated protein production in vitro. When these acts are taken together, they effectively encourage bone repair. Clipet *et al.*[39] found that PRF conditioned media stimulated gene expression in osteoblasts, as well as the expression of osteopontin, osteocalcin, and late osteogenic markers, in an in vitro investigation, implying that PRF may be effective in accelerating tissue healing and bone regeneration. It is also stated that PRF membrane protects the surgical site, promotes soft tissue healing, and acts as a "biological connector" between different elements of the graft and acts as a matrix that supports neo-angiogenesis, stem cell capture, and migration of osteoprogenitor cells to the graft's centre when its fragment mixes with graft material[40].

Despite the fact that both combination are techniques showed promise in terms of improving periodontal regeneration. However, when compared to HA+CM treatment for mandibular molar furcation abnormalities, the combination of HA+PRF membrane exhibited a greater improvement in IBD depth.

Limitations of the study were small sample size long term analysis of results is required to determine the stability of treatment outcomes. Present clinical results in term of regeneration were not supported by histological outcomes.

5. Conclusion

In terms of clinical outcomes and cost, PRF membrane may be a preferable option for the treatment of Grade II furcation problems. To confirm the effectiveness of PRF membrane and its combination with other regenerative materials in the management of furcation and other osseous abnormalities, more longitudinal studies with long-term follow-up are needed.

Consent: Participants were informed in their vernacular language regarding the procedure, and written informed consent was obtained and preserved by authors.

Ethical Approval: The study protocol was complied with the Helsinki declaration and approved by the Institutional Ethical Committee.

Competing Interests: Authors have declared that no competing interests exist.

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