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Regenerative potential of a combination of PRF and bioactive glass for the treatment of horizontal bone defects in stage II and stage III periodontitis: a split-mouth randomized controlled trial

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Abstract

Objective: The aim of the study was to evaluate the efficacy of calcium phosphosilicate bioactive glass in combination with Platelet Rich Fibrin (PRF) in the treatment of horizontal bone defects in stages II and III periodontitis.

Materials and Methods: The randomized controlled trial included 51 sites of horizontal bone defects from 4 patients with stages II and III periodontitis. The three treatment groups were: Group A - open flap debridement (OFD) + bone graft +PRF gel; Group B - OFD+ bone graft+ PRF gel covered by PRF membrane; Group C - OFD alone. The clinical and radiographic parameters were recorded at baseline and 9 months post-surgery.

Results: All the three groups showed statistically significant improvement in clinical and radiographic parameters, except for defect width at 9 months post-surgery.

Conclusion: The findings of the present study allows to conclude that the use of PRF in combination with bone graft can serve as a promising therapeutic approach for the treatment of horizontal bone defects, resulting in improvement of both clinical and radiographic parameters.

Keywords:* *Periodontitis. Platelet rich fibrin. Debridement. CBCT.

Introduction

Alveolar bone destruction mediated by the host immune and inflammatory response to the microbial challenge is a hallmark of advanced stages of periodontitis, and its management presents a major clinical challenge in the treatment of periodontal diseases. Among the various patterns of bone loss seen in periodontitis, horizontal and vertical bone loss are the most prominent forms (Jayakumar *et al*., 2010). The literature states that although horizontal bone loss is the most prevalent form of bone loss (96.8%), there are still very few treatment modalities (3.2%) credited to its successful regeneration (Jayakumar *et al*., 2010).

Bone regeneration in horizontal defects presents many challenges, which makes open flap debridement (OFD) the most appropriate therapy (Wikesjo *et al*., 1999). However, the advent of various biological approaches and biomaterials has revived the research potential of horizontal bone regeneration in periodontics.

The bioactive properties of bioactive glass have caused a revolution in healthcare, with successful attempts for hard tissue regeneration in medicine and dentistry. Bioactive glass when used as a bone regenerative material is considered to be the second best bone graft material, when compared to autografts (Rahaman *et al*., 2011). Bioactive glass (45S5) has been shown to enhance new bone formation *in vivo* (Wheeler *et al*., 1998). Bioactive glass can be osteoconductive and osteoinductive, because it supports new bone growth along the bone/implant interface, as well as within the implant away from the bone/implant interface. The beneficial effects of small concentrations of 45S5 bioactive glass for stimulating angiogenesis has been shown in several recent studies. The ability of a bioactive glass to induce angiogenesis can provide a robust alternative approach to the use of expensive growth factors for stimulating neovascularization of engineered tissues. Despite its brittleness, bioactive glass

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has a unique set of properties, such as the ability to degrade at a controllable rate and convert to an HA-like material, to bond firmly to hard and soft tissues, and to release ions during the degradation process. These ions are known to have a beneficial effect on osteogenesis and on angiogenesis, and recent results indicate that they may also have a beneficial effect on chondrogenesis (Gorustovich *et al*., 2010). Not only does it have an osteoconductive property, but it also exhibits osteostimulatory effect, by merging with the neighboring tissues (Cannillo *et al*., 2022)

There are many studies (Joseph *et al*., 2014; Debnath *et al*., 2018) that have evaluated the potential of platelet rich fibrin (PRF) in regenerating horizontal bone defects. But the lack of rigidity and space maintenance by PRF may have a negative impact on horizontal bone regeneration (Liu *et al*., 2019). However, this disadvantage of PRF can be overcome by the addition of a bioactive glass to freshly prepared PRF, thereby improving its handling properties (Chandradas *et al*., 2016). This can eventually result in rapid healing and bone augmentation, due to its dense fibrin network and rich growth factors.

Hence, the aim of the present study was to evaluate the efficacy of calcium phosphosilicate bioactive glass in combination with two forms of autologous platelet rich fibrin, in comparison with open flap debridement, in the treatment of horizontal bone defects in stages II and III periodontitis.

Materials and Methods

Study design

A prospective, split-mouth randomized, controlled trial with a nine-months follow-up was conducted to evaluate the clinical and radiographic outcomes of the three treatment groups. All procedures were performed according to the Helsinki declaration of 1975 (revised in 2013), and were approved by the institutional ethical committee. The study was registered under the clinical trials registry of India with registration number: CTRI/2021/02/031359.

Participants

Patients were recruited from the outpatient Department of Periodontology and Oral Implantology at Vydehi Institute of Dental Sciences and Research Centre, Bangalore/India. All participants signed an informed consent agreement after deciding to enroll. The study was conducted between August 2020 and December 2021. As proposed by the VII European Workshop on Periodontology, the modified Consolidated Standards of Reporting Trials (CONSORT) guidelines were applied (Figure 1).

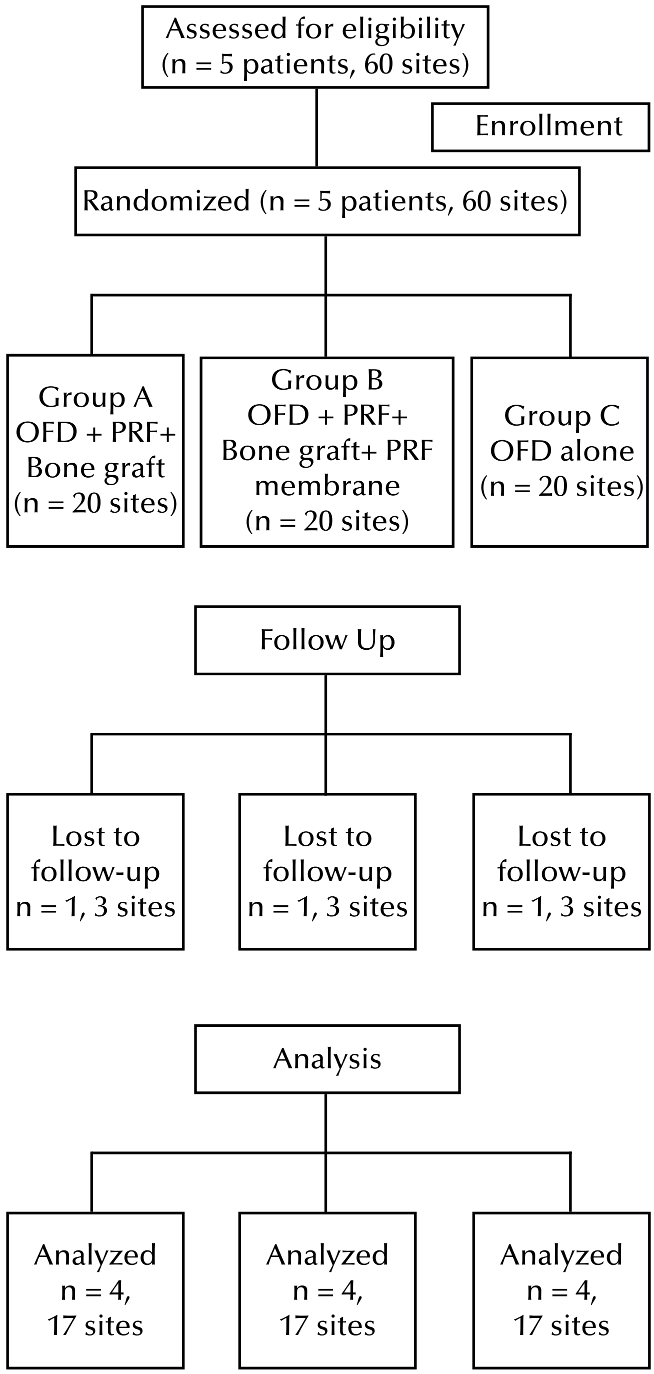

Figure 1. Study flowchart, following the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

The required sample size was 17 sites of horizontal bone defects per treatment group, considering an alpha error of 0.05 and 80% power of the study. Considering a drop out of 20%, the sample size was rounded up to 20 sites. Since the study involved three groups, a total of 60 sites was calculated (20 in each group).

Inclusion and exclusion criteria

Patients in the age group of 30-60 years diagnosed with stages II and III periodontitis, according to 2017 World Workshop on the Classification of Periodontal and Peri-implant diseases and conditions, were evaluated for the study. Medically compromised patients, patients who underwent periodontal surgery in the past one year, smokers, pregnant and lactating women were excluded from the study. All patients received steps 1 and 2 of periodontal therapy (Sanz *et al.*, 2020). Sites with horizontal bone loss of 5mm or more in premolars and molars, with a probing depth of 5mm to 8mm and a bone loss of 5mm or more from the cementoenamel junction (CEJ) to the alveolar crest in CBCT (Cone Beam Computed Tomography) following steps 1 and 2 of periodontal therapy were included in the study. The study coordinator (NKJ) randomly allocated the participants to one of the three treatment groups, according to the lottery method of randomization: Group A – open flap debridement (OFD) + bone graft + PRF gel; Group B – OFD + bone graft + PRF gel covered by PRF membrane; Group C – OFD alone. This sequence was placed in sealed opaque envelopes, each one containing a treatment modality, ensuring allocation concealment. All clinical and radiographic measurements were performed by one calibrated examiner (SSe), who was blinded for treatment allocation. The surgical procedure was performed by another calibrated examiner (SS), under local anesthesia using 2% lignocaine hydrochloride containing adrenaline at a concentration of 1:200000. Patients were required to maintain adequate oral hygiene and control of inflammation in the whole dentition, as demonstrated by a full-mouth plaque score (FMPS) <20% (Bertoldi *et al.*, 2017). Surgical procedures were initiated only when the plaque score and bleeding score were below 20%, and oral hygiene reinforcements were repeated to maintain it throughout the study period.

Baseline clinical examination

Probing pocket depth (PPD) and clinical attachment level (CAL) were recorded using UNC-15 periodontal probe. An acrylic stent was made to fit on the occlusal/incisal surfaces of the teeth, and the measurements were made using an UNC-15 probe, by placing it in the groove made on the stent (Figure 2).

Figure 2. Acrylic stent used for the measurement.

Radiographic examination

CBCT was taken to obtain three-dimensional visualization of the horizontal defects. In CBCT, defect depth was measured from the CEJ to the alveolar crest (CEJ-CAB); and the width of the bone, was measured at the thinnest portion between the buccal/lingual and palatal plate (DW) in axial tomogram. All the clinical and radiographic measurements were performed by one calibrated examiner (SSe) who was blinded for treatment allocation.

Surgical procedure

The surgical procedure was performed under local anesthesia using 2% lignocaine hydrochloride containing adrenaline at a concentration of 1:200000 (LOX*2% ADRENALINE). Following anesthesia, crevicular incisions and interdental incisions were made. A full thickness mucoperiosteal flap was raised. The mesiodistal and apicocoronal flap extensions were placed in such a way as to completely expose the horizontal defects and preserve the marginal gingiva and interdental tissue, followed by meticulous debridement and root planing. The deepest defect from the interproximal area was measured from the CEJ to the base of the defect, and CEJ served as a fixed reference point.

Intramarrow penetration was done for patients of all groups using a contra-angled micromotor handpiece and a round carbide bur (1-mm diameter) to reach the marrow space. Multiple perforations were performed, not closer than 1mm from each other, and deep enough to obtain bleeding. Pre-suturing was done prior to the placement of the graft (Perioglas mixed with PRF gel), to prevent the dislodgement of graft material. The sites in Group A (Experimental group 1) received PRF mixed with bone graft (Figure 3), and in Group B (Experimental group 2) received PRF mixed with bone graft covered by PRF membrane (Figure 4) (obtained by pressing the PRF clot between two pieces of gauze). Choukroun *et al*. (2001) developed a protocol of platelet-rich fibrin (PRF) gel to accumulate platelets along with released cytokines in a fibrin clot. Choukroun's protocol uses centrifuged natural blood without any anticoagulant. To make a PRF membrane (PRFM), moist or dry gauze was used to compress the PRF clot (Dohan *et al*., 2006). The bone graft used was a synthetic absorbable osteoconductive bone graft substitute composed of calcium phosphosilicate bioactive glass (Perioglas™, NovaBone Products, LLC, Alachua, FL 32615, USA). The flaps were repositioned to their presurgical level and sutured with 3-0 silk sutures. Periodontal pack was placed to cover the surgical areas.

Figure 3. PRF mixed with bone graft placed in the defect.

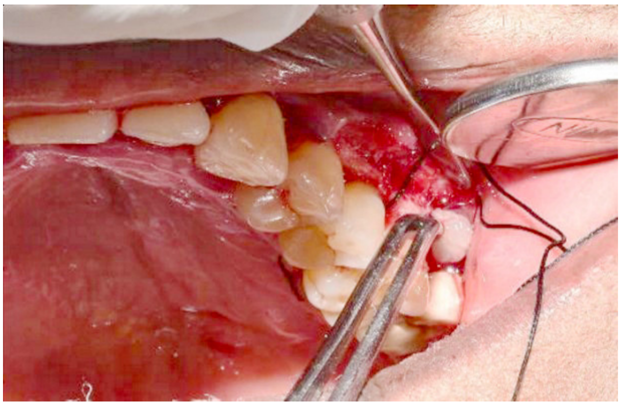

Figure 4. PRF mixed with bone graft covered by PRF membrane.

Preparation of PRF

PRF was prepared according to the protocol proposed by Choukroun *et al*. (Dohan *et al*., 2006). Blood collection and preparation of PRF was delayed until adequately exposing the bone defects, so that the prepared PRF could be used instantaneously without the need for any storage. 10ml of blood was collected from the patient by venepuncture of the right antecubital vein. Blood was collected in sterile glass tubes without any anticoagulants, and immediately centrifuged on a table top centrifuge at 3000 rpm for 10 minutes (REMI NEYA 4 bench top centrifuge). This resulted in the separation of three basic fractions, due to different densities: red blood cells (RBC, bottom layer), PRF (middle layer) and platelet poor plasma (PPP, top layer). The PPP was aspirated and discarded, and the PRF was isolated from the underlying RBC layer by using sterile stainless steel scissors. The PRF gel thus obtained was mixed with bone graft with the help of a spatula and placed in the decorticated area, as well as at the level of the defect.

Post-operative care

Post-surgically, the patients were prescribed antibiotics (Novamox 500mg -Amoxicillin 500mg, *tid* for 5 days) and analgesics (Diclomol -Diclomol-Diclofenac 50mg, paracetamol 325mg) for 5 days along with 0.2% chlorhexidine gluconate (Clohex ADS) rinse twice daily for one week. Sutures were removed after 10 days. The patients were recalled at regular intervals for oral hygiene reinforcement and prophylaxis.

Statistical analysis

At the end of nine months, one patient with nine sites of horizontal bone loss was lost to follow up due to the prevailing Covid-19 pandemic. Therefore, data from four patients, with 51 sites (17 in each group) were available at nine months post-surgical re-evaluation.

The frequency distribution for categorical data was expressed in terms of number and percentage, whereas for continuous data, it was expressed in frequency, mean and standard deviation (SD). One-way ANOVA test was used to compare the mean values of clinical and radiological parameters at baseline and nine months post-surgery. Tukey's HSD *post-hoc* analysis was used to compare the mean clinical and radiological parameters between the groups at baseline and nine months post-surgery. Student paired *t*-test was used to compare the study parameters within the group at baseline and nine months post-surgery. The level of significance was set at $p<0.05$. Statistical Package for Social Sciences (SPSS) for Windows, version 22.0 released 2013 (Armonk, NY: IBM Corp.) was used to perform statistical analyses.

Results

At baseline, no statistically significant differences were observed between the experimental and control sites, with respect to probing pocket depth, clinical attachment level, distance from the CEJ to the CAB, and defect width ($p > 0.05$).

At nine-months reevaluation, all the groups showed statistically significant improvement in all the clinical and radiological parameters, except defect width. Group A and Group B showed statistically significant improvements in both clinical and radiographic parameters (Figure 5 and Figure 6), except for defect width (Figure 7), when compared to Group C, which was the control group. When intergroup comparison was made between Group A and Group B, there was no statistically significant improvements (Table 1).

Defect fill percentage (DF %)

The DF percentage was calculated based on the linear radiographic depth of the defect obtained from the CBCT. The defect fill was calculated according to the following formula: Baseline defect depth – Defect depth at nine months / Baseline defect ×100.

When intergroup comparison was done for defect fill percentage, Groups A and B were statistically significant, compared to Group C. Groups A and B showed no statistically significant differences in defect fill percentage (Table 1).

Figure 5. Pre-operative CEJ-CAB distance.

Figure 6. Nine months post-operative CEJ-CAB distance.

Figure 7. Bone width at the thinnest portion between the buccal/lingual and palatal plate (DW) in axial tomogram.

Table 1. Comparison of mean values of the study parameters at the nine months interval, using one-way ANOVA test followed by Tukey's HSD *post-hoc* analysis.

Parameters	Group A [n=17]		Group B [n=17]		Group C [n=17]		*p*-value	Tukey's *post-hoc* Analysis		
	Mean	SD	Mean	SD	Mean	SD		A *vs.* B	A *vs.* C	B *vs.* C
PPD	3.09	0.77	2.92	0.49	3.69	0.68	0.003*	0.74	0.03**	0.004**
CAL	3.91	0.84	3.73	0.71	4.70	0.59	0.001*	0.74	0.007**	0.001**
CEJ-CAB	4.32	0.83	4.02	0.69	5.01	0.58	0.001*	0.45	0.02**	0.001**
Defect width	8.93	1.19	8.76	1.01	9.08	1.36	0.74	0.91	0.93	0.72
Defect fill (%)	36.35	4.94	41.59	4.77	23.99	8.48	<0.001*	0.08	<0.001**	<0.001*

* Statistically significant (intragroup). ** Statistically significant (intergroup). *p*-value < 0.001. PPD = Probing pocket depth. CAL = clinical attachment level. CEJ-CAB = distance from the cementoenamel junction to the crestal alveolar bone.

Discussion

The purpose of this randomized split-mouth controlled trial was to evaluate the efficacy of calcium phosphosilicate bioactive glass in combination with autologous platelet rich fibrin in the management of horizontal bone defects. A split-mouth design was chosen as it would allow a better assessment of how the same patient would respond to different treatment modalities. Various studies have evaluated the regenerative potential of PRF combined with decalcified freeze-dried bone allograft (DFDBA) for the treatment of intrabony defects, and obtained positive outcomes (Agarwal *et al*., 2016, Yajamanya *et al*., 2017). Previous studies have evaluated the effect of PRF alone in both gel and membrane form in the treatment of horizontal bone defects (Joseph *et al*., 2014, Debnath *et al*., 2018). Regarding the disadvantages of PRF in terms of lack of rigidity and space maintenance, we decided to combine PRF with a bone graft to render better handling property for PRF.

The intergroup comparison of the clinical and radiologic parameters at baseline showed no statistically significant differences (Table 2). At nine months post-surgery, all the groups showed significant reduction in probing depth and gain in clinical attachment level, compared to baseline. The improvement in clinical parameters was more pronounced in Groups A and B, compared to Group C. The reduction of 3.11 mm in the mean PPD and the CAL gain of 2.96 mm were similar to a study conducted by Joseph *et al.* in 2014, with PPD reduction of 1.7 mm and CAL gain of 1.7 mm, in which PRF was used in gel and membrane forms to treat horizontal bone defects. The results were also correlated to a study conducted by Debnath *et al.* in 2018, in which the reduction in mean PPD was 1.58 mm and CAL gain was 1.5 mm in the experimental group where PRF matrix was used for the treatment of horizontal bone defects. The improvement in clinical parameters in Groups A and B in the present study can be attributed to the use of a combination of PRF and bone graft. As stated by Chang and Zhao *et al.* in 2011, PRF has a unique structure that may act as a vehicle for carrying cells that are essential for tissue regeneration. Though the differences between Groups A and B were not statistically significant, the parameters showed greater improvement in Group B, in which PRF membrane was used in addition to PRF gel and bone graft. The PRF membrane could have served as a resorbable membrane for guided tissue regeneration, acting as structural support and a mechanical barrier to create space around the defects, preventing epithelial growth into the defect area, thus preventing the formation of long junctional epithelium, permitting periodontal regeneration (Ozdemir *et al*., 2012). The radiological parameters evaluated using CBCT were the defect depth from CEJ to CAB and the defect width between the buccal and

Table 2. Comparison of mean values of study parameters at baseline period using one-way ANOVA test.

Parameters	Group A [n=17]		Group B [n=17]		Group C [n=17]		*p*-value
	Mean	SD	Mean	SD	Mean	SD	
PPD	5.81	1.02	6.03	0.74	5.56	0.52	0.23
CAL	6.40	1.01	6.69	0.81	6.41	0.60	0.51
CEJ-CAB	6.75	1.02	6.87	0.81	6.62	0.56	0.68
Defect width	8.84	1.21	8.70	1.00	8.98	1.36	0.80

PPD = Probing pocket depth. CAL = clinical attachment level.

palatal/lingual plates. At nine months post-surgery, all groups showed an increase in the defect fill. A defect fill of 36.35%, 41.59%, 23.99% was obtained in Groups A, B and C, respectively. The defect fill was significantly enhanced in Groups A and B, compared to Group C. This result was comparable to the result observed in the study conducted by Debnath *et al.* in 2018, in which the use of PRFM had resulted in a defect fill of 45.09%. The difference of 3-4% may be due to the difference in baseline parameters recorded in the two studies. The results were in contradiction to a study conducted by Joseph *et al.* in 2014, in which the use of PRF did not bring any significant radiographic improvement in the bone levels at nine months post-surgery. This could have been due to the use of standardized intraoral radiographs in the study, for assessing bone level, as opposed to advanced radiographic techniques. Assessment of radiological parameters using CBCT in the present study helped to obtain more precise bone levels, due to the high diagnostic accuracy of CBCT. With the use of CBCT, it was possible to assess the bone defects in three-dimensional view, thereby also including defect width, as opposed to the previous studies, in which only the height of the defect was taken into account. The objective of using CBCT in the present study was to obtain more precise radiographic evaluation, due to the high sensitivity and diagnostic accuracy of CBCT, as evidenced by Bagis *et al.* (2015).

The improvement in radiological parameters can be attributed to the additional benefits of PRF in combination with bone graft, in which PRF mixes with the fragments of bone and functions as a biological connector between the different elements of graft, acting as a matrix that supports neoangiogenesis, capture stem cells, and migration of osteoprogenitor cells to the center of graft. The graft particle size of Perioglas ranges from 90 to 710 μm. The glass particles usually have a size of 90 to 170 μm, which affects their resorption rate. Particles smaller than 150 μm readily degrade as orthosilicic acid is released during the formation of the gel layer. Osteoclasts, once incorporated in the growing bone, break down larger particles (Gosain *et al.*, 2004) resulting in a more extended period of resorption and stronger bone (Ducheyne *et al.*, 1999) . One of the major disadvantage of PRF membrane is the lack of rigidity, which may cause the membrane to collapse over the bone and root surface, thus limiting the space that is necessary for clot maturation. In the present study, PRF gel mixed with bone graft was used to support the PRF membrane that separated the gingival flap from contacting with the bone surface. When the bioactive glass was mixed with the PRF clot, the serum extracted from the PRF clot formed a matrix that shrank upon mixing, thus forming a less bulky matrix, improving the material's handling property when placed in horizontal bone defects (Simon *et al.*, 2018). This serum, called hyperacute serum, has higher cell proliferative effect on bone marrow mesenchymal stem cells (MSCs), osteoblasts and osteoarthritic chondrocyte cells. Taking into consideration that the present study included horizontal defects, intramarrow penetration was done for patients of all groups using a contra-angled micromotor handpiece and a round carbide bur (1-mm diameter) to reach the marrow space. Multiple perforations were performed, not closer than 1mm from each other and deep enough to expose the cancellous bone.

Another attempt of increasing bone regeneration performed in the present study was the technique of intramarrow penetration (IMP), which was done with the help of airotor handpiece with round bur. This technique was performed with the intention of exposing the cancellous bone, thereby enhancing the healing process by promoting bleeding and allowing progenitor cells and blood vessels to reach a bone-grafted site more readily. Performing IMP in the surgical site creates a close spatial correlation between angiogenesis and bone formation. The vessel-rich medullary opening space would facilitate capillary sprouting and enhance vascular access into the surgical site. A local increase in bone morphogenetic proteins and other growth factors from the injured cortical surface, endosteal area, and wounded vessels area can enhance further new bone formation (Majzoub *et al.*, 1999). This was substantiated by a study conducted by Crea *et al.* in 2014, which reported that the addition of IMP to an OFD procedure used to treat intrabony defects results in statistically and clinically significant enhancement of both clinical and radiographic outcomes. Other studies (Joseph *et al.*, 2014; Debnath *et al.*, 2018) have also shown the positive effect of intramarrow penetration in horizontal bone defects. The present study also compared the defect width (distance between the buccal and palatal/lingual plates), which was facilitated by the three-dimensional view in CBCT. At nine months post-surgery, no significant improvement in the defect width was observed, compared to the baseline. One possible reason for no improvement can be due to the horizontal nature of bone loss, wherein the crestal bone height gets reduced without much effect on the bucco-lingual or bucco-palatal dimension.

One limitation of this study was the absence of a treatment group using PRF alone. However, this was not considered, due to the availability of studies conducted using PRF alone in the management of horizontal bone defect. Since PRF is known to improve the wound healing properties, a wound healing index could have been used in the study one week after surgery, to evaluate the post-operative pain and healing characteristics of the wound.

Conclusion

The ultimate goal of any periodontal therapy is a successful regeneration of lost alveolar bone, thereby maintaining the health of the tooth and periodontium. Horizontal defects being more prevalent than vertical defects, has now succeeded in gaining researcher's attention. But the published articles related to successful treatment modalities for regeneration of horizontal defects are still scarce.

Within the limitations of this study, it can be concluded that the use of PRF in combination with calcium phosphosilicate bioactive glass significantly improved the clinical and radiological parameters in patients with horizontal defects in stages II and III periodontitis. The use of autologous growth factor delivery system in the form of PRF offers a new dimension in the management of horizontal bone defects in periodontitis. The additional use of bone graft has further assisted in better handling property of PRF, as well as improved the clinical and radiological parameters. Thus, the use of PRF in combination with bone graft can be adopted as a potential treatment modality in the management of horizontal bone defects. This study seeks to generate greater curiosity among researchers to find a regenerative treatment modality in an otherwise pervasive periodontal challenge.

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