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RESEARCH ARTICLE

PRF and PRP in periodontal regeneration- A comparative clinical and radiological study

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Abstract

This randomized, longitudinal interventional study was carried out on 17 systemically healthy subjects to evaluate and compare, clinically and radiographically, the efficacy of a combination of Platelet-rich-Fibrin and Bioactive Glass, Platelet-rich-Plasma and Bioactive Glass and Bioactive Glass alone in the treatment of periodontal endosseous defects.

Materials and methods

30 intrabony defects were selected and divided into three groups randomly by the coin toss method. Group I received bioactive glass (PerioGlas®) only. Group II subjects were treated with a combination of Platelet- rich Plasma and PerioGlas® and Group III, with a combination of Platelet- rich Fibrin and PerioGlas®. Clinical and radiological parameters were assessed at baseline and 3, 6 and 9 months postoperatively. The results obtained were statistically analyzed.

Results

The difference in Probing Pocket Depth (PPD) between the Groups I and III and Groups II and III were significant at the end of 9 months. The difference in Clinical Attachment Level (CAL) at the end of 9 months was significant between the Groups I and III and Groups II and III. The difference in depth of defect between the Groups I and II, Groups I and III were significant at the end of 9 months.

Conclusion

Platelet-rich Fibrin and Platelet-rich Plasma appear to have nearly comparable effects in terms of periodontal regeneration, with Platelet-rich Fibrin displaying slightly superior effect in comparison to Platelet-rich Plasma, which in turn displayed a superior efficacy in comparison to the bioactive glass synthetic bone graft particles alone.

Clinical significance

The results from the study indicate that Platelet-rich-Plasma (PRP), Platelet-rich-Fibrin (PRF) in combination with bioactive glass synthetic bone graft particles and bioactive glass synthetic bone graft particles alone are efficacious materials in the treatment of periodontal endosseous defects and can be used as effective regenerative materials.

Introduction

Periodontal disease is characterized by the presence of gingival inflammation, periodontal pocket formation, and loss of connective tissue attachment and alveolar bone around the affected teeth (Hanna et al, 2004).Periodontal therapy employs therapeutic modalities that helps to arrest the disease progression and to regenerate the lost tissue and thus protects and maintains the patient's natural dentition (Tozum et al,2003). Periodontal regeneration is defined as the reproduction or reconstitution of a lost or injured part so that form and function of lost structures are restored (GPT,1992). Periodontal surgical procedures have focused on the elimination of hard and soft tissue defects (i.e., probing depths and osseous defects) by regenerating new attachment (Froum et al, 1998). Several reconstructive modalities have demonstrated significant gain of clinical attachment and at least partial resolution of an associated bony defect (Becker et al,1999).

In periodontal regeneration, the lost tissues are restored to their original architecture and function by reiterating the crucial wound healing events associated with their development (Sharma et al,2011a). Polypeptide growth factors (PGFs) are believed to be able to achieve the recapitulation of the events leading to regeneration and hence, a great interest in these factors has been generated. PDGF and TGF-β have been shown to promote cell growth, differentiation, and periodontal regeneration (Yilmaz et al,2011).

Platelet-rich plasma (PRP) is an autologous source of PDGF and TGF-β that is obtained by sequestering and concentrating platelets by gradient density centrifugation. It has been demonstrated to induce healing and regeneration of tissues, including those in the periodontal area (Tozum et al, 2003). PRF was first developed in France by Choukroun et al and the biochemical analysis of PRF has shown that this biomaterial consists of an intimate assembly of cytokines, glycanic chains, and structural glycoproteins enmeshed within a slowly polymerized fibrin network and these have been known to have synergetic effects on healing processes (Dohan et al, 2006a).

It has been shown that platelet concentrate, in adjunction with bone graft material makes it possible to amplify the graft volume without injuring the maturation quality in new bone (Choukroun et al, 2006). The bioactive glasses are alloplastic graft materials that have been applied to dentistry in the treatment of bone defects, ridge preservation and periodontal bone defects (Subbaiah et al, 2011).

A paucity of studies comparing the effects of Bioactive Glass alone and in combination with Platelet-Rich-Plasma and Platelet-Rich-Fibrin on bone regeneration was noted and hence this study was designed to evaluate and compare, clinically and radiographically, the efficacy of Platelet-Rich-Fibrin and Bioactive Glass, Platelet-Rich-Plasma and Bioactive Glass and Bioactive Glass alone in the treatment of periodontal endosseous defects.

MATERIALS AND METHODS

Subjects and study groups

A randomized, longitudinal interventional study involving a total of 17 systemically healthy subjects, contributing to a total of 30 surgical sites was designed and conducted on a study population selected from the subjects visiting the out-patient section of the Department of Periodontics, D A Pandu Memorial R V Dental College, Bangalore. The ethical clearance for the study was obtained from the ethical committee and review board of the institution.

Patients aged between 20-55 years, who were systemically healthy and had no contraindications for periodontal therapy met the inclusion criteria. A patient was not considered eligible if gingival index score was >2.1. All patients were non-smokers. Two and combined 3-wall intrabony periodontal defects with a probing pocket depth (PPD) ≥5 mm, radiographic defect depth ≥3 mm were included in the study.

The 30 surgical sites were identified and divided into 3 groups; Group I, Group II, Group III. The coin toss method was used to randomize the patients to receive the various treatment options.

The groups were:

Group I (n=10): Those to be treated with bioactive glass (PerioGlas®) only

Group II (n=10): Those to be treated with a combination of Platelet rich plasma and PerioGlas®

Group III (n=10): Those to be treated with a combination of Platelet rich fibrin and PerioGlas®

Clinical and Radiographic Assessments

Oral hygiene status was assessed using Plaque Index (Sillness and Loe (1964)) and Gingival index (Loe and Sillness (1963)). Probing pocket depth (PPD), clinical attachment level (CAL), and marginal recession (GR) were measured to the nearest millimeter with a calibrated periodontal probe using an occlusal stent as a reference point for probe placement. Occlusal stents for positioning measuring probes were fabricated with cold-cured acrylic resin on a cast model obtained from an alginate impression. Measurements were recorded from (Fig 1):

- Stent to cementoenamel junction (A)
- Stent to gingival margin (B)
- Stent to deepest probing depth at test sites (C)

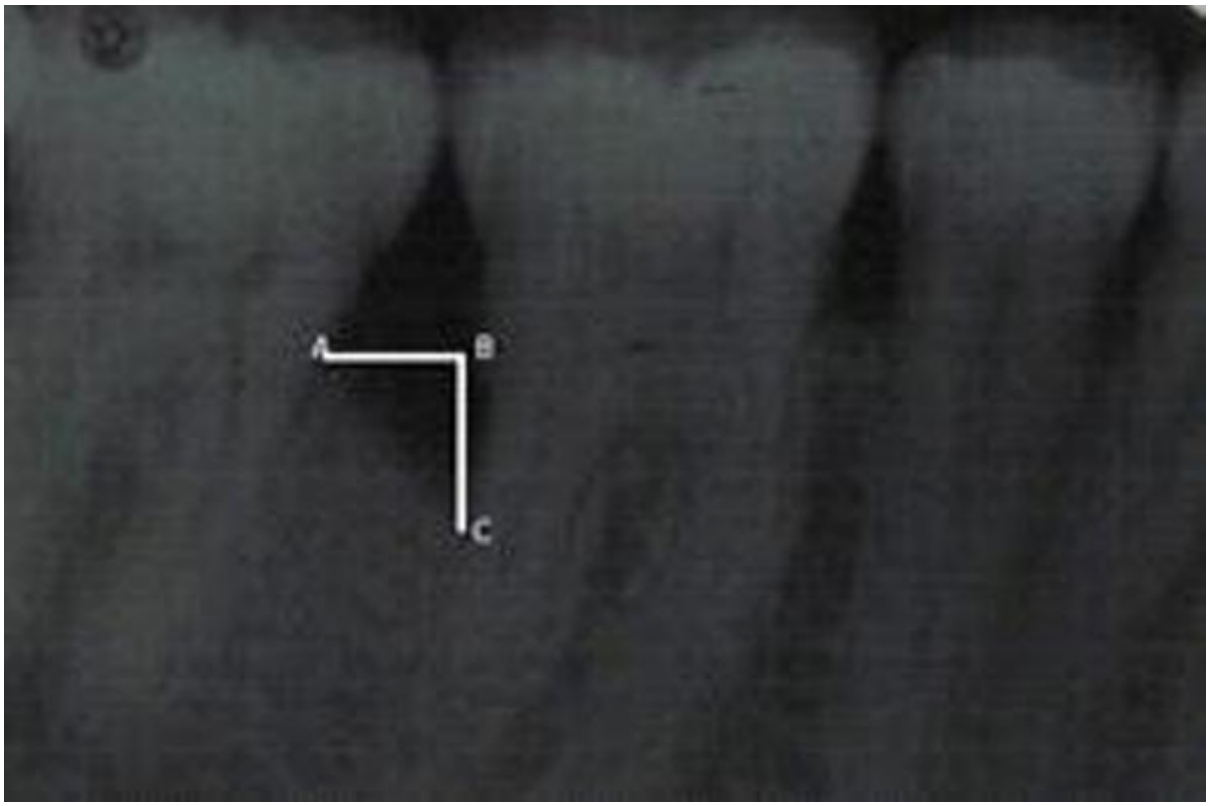
Calculation of the parameters:

Probing pocket depth (PPD) = Stent to deepest probing depth at test sites (C) - Stent to gingival margin (B)

Clinical Attachment level (CAL) = Stent to deepest probing depth at test sites (C) - Stent to cementoenamel junction (A)

Gingival recession= Stent to gingival margin (B) - Stent to cementoenamel junction (A)

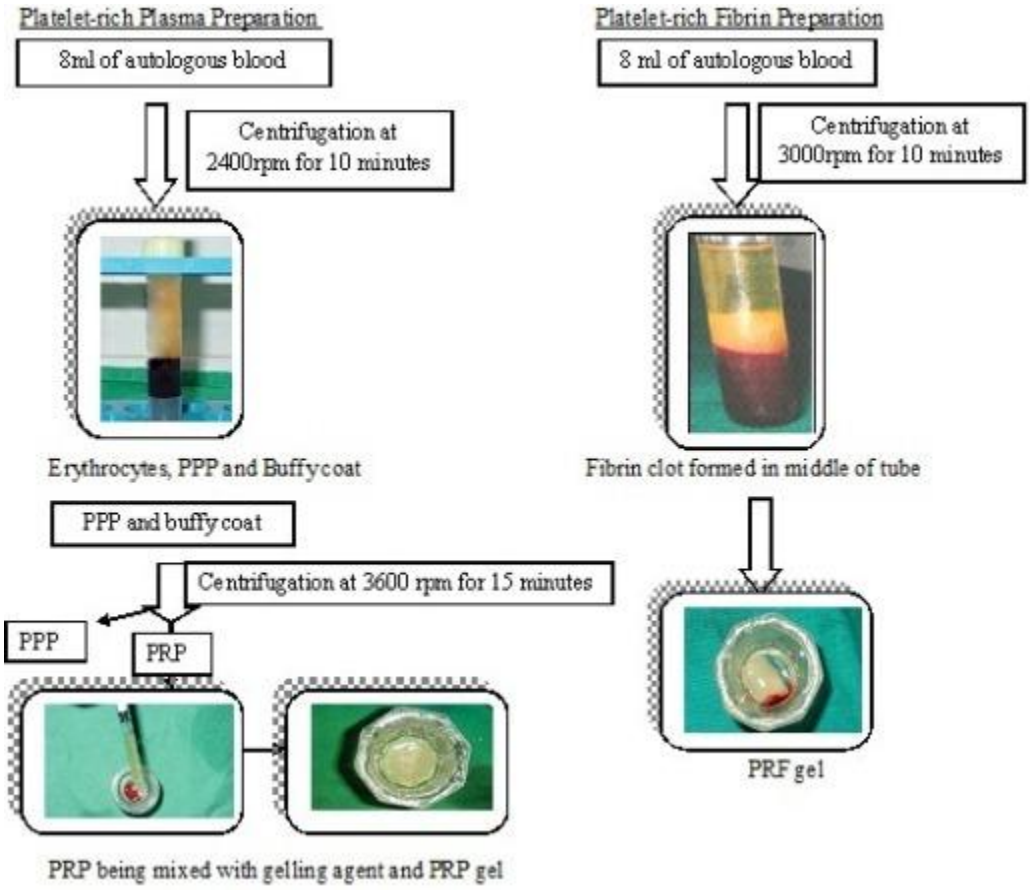
Intraoral periapical radiographs were taken using long cone paralleling technique with a radiographic grid in position. The depth of the bone defect was assessed to the closest 0.5 mm on the intraoral periapical radiograph taken with the radiographic grid in position. A horizontal line was drawn projecting from the point on the bone crest designated as 'A'. The horizontal line was drawn perpendicular to the long axis of the root surface of the tooth associated with the vertical defect and the point of contact of the horizontal line with the root surface was designated as 'B'. A vertical line was then drawn from 'B' to the most coronal level along the root surface where the periodontal ligament space was considered to have a normal width; the point was designated as 'C'. The vertical dimension between 'B' and 'C' was measured to assess the bone level at the baseline evaluation and was designated as BC0 (Fig 2).



PRP and PRF preparation

Platelet Rich Plasma (PRP) was prepared using the following procedure; The left antecubital fossa was swabbed with an alcohol swab and a cuff was used to apply pressure above the fossa. A 10 ml syringe was used to draw 8 ml of blood and immediately transferred into a test tube containing 1.5 ml of citrate anticoagulant solution (Anticoagulant citrate dextrose solution). The sample tube was then spun in a standard centrifuge for 10 minutes at 2400 rpm to produce Platelet Poor Plasma (PPP) (Fig 3). The Platelet Poor Plasma (PPP) was taken up into a syringe with a long cannula. A second centrifugation (15 minutes at 3600 rpm) was performed to concentrate the platelets. The second supernatant was also taken up by a long cannula. This was PRP, which was used for the surgical procedure (Tozum et al, 2003). At the time of the application PRP was combined with calcium gluconate to facilitate plasma coagulation (Fig 3). The gel thus obtained was then mixed with PerioGlas®.

The PRF was prepared following the protocol developed by Choukroun et al (2006). The patient's blood samples were drawn prior to the surgery following the same procedure as that for PRP preparation. Immediately after the blood draw, the dried monovettes (without anticoagulant) were centrifuged at 3000 rpm for 10 minutes in the table top centrifuge. A structured fibrin clot formed in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top (Platelet Poor Plasma-PPP) (Fig 3). PRF was separated from red corpuscles at the base, preserving a small red blood cell (RBC) layer, using a sterile tweezers and scissors just after removal of Platelet Poor Plasma (PPP) and then transferred onto a sterile dappen dish (Fig 3). The gel obtained was mixed with PerioGlas®



Surgical Procedure

After local anesthesia, an intrasulcular incision aiming to preserve the papillae was performed. Mucoperiosteal buccal and lingual access flaps were then reflected. Granulation tissue adherent to the alveolar bone was removed to provide full access and visibility to the root surfaces. Any subgingival calculus was removed gently by using hand instruments. Then, PerioGlas® alone, PRP/PerioGlas® or PPP/PerioGlas® combination was packed into the defects. Finally, the flaps were replaced and sutured appropriately with a 3-0 silk material using interdental suture technique. After a healing period of 10 days, the sutures were removed.

Postoperative Care

All patients received systemic antibiotic therapy for a period of 5 days postoperatively (amoxicillin 500 mg three times per day for 5 days). In addition, all patients were advised to avoid tooth brushing and hard chewing in the surgical areas and to rinse twice daily with a 0.2% solution of chlorhexidinedigluconate for 2 weeks. Recall appointments were scheduled every second week during the first 2 months after the surgical procedure, and all patients were recalled once a month for the remaining observation period.

Post-Surgical Evaluation and Review

Gingival Index (GI) and Plaque Index (PI) were re-evaluated at 3 months, 6 months and 9 months. Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Gingival Recession (GR) were also re-evaluated at 3 months, 6 months and 9 months using the previously used acrylic stents to provide a reproducible insertion axis.

Radiographic parameters:

The vertical dimension between ‘B’ and ‘C’, measured to assess the depth of the defect at 3 months, 6 months and 9 months, were designated as BC3, BC6, BC9 respectively. The bone fill at the end of 9 months in each group was obtained by subtracting BC9 from BC0.

Statistical analysis

Statistical analysis was done using the SPSS software. The intergroup comparisons were performed used Kruskal-Wallis chi-square test. The within group comparison was performed using Wilcoxon signed rank test.

RESULTS

35 advanced chronic periodontitis patients were screened against the criteria listed for eligibility for this study. 13 patients did not meet the inclusion criteria and five patients refused to participate in the study. The remaining 17 patients received the intended treatment. No patients were lost to follow-up and returned for clinical and radiographic evaluation at 9 months. Clinical evaluation of post-surgical healing revealed a good soft tissue response to the combinations with no adverse complications. Both groups presented similar baseline characteristics in terms of PPD, GR, CAL, plaque index, gingival index.

All patients maintained a good level of oral hygiene and gingival status throughout the recall periods. Intergroup differences were found to be insignificant (P >0.05) in terms of plaque index and gingival index (Table 1 and Table 2).

At 9 months, all the groups presented a significant improvement in terms of PPD reduction and CAL gain (Table 3 and Table 4). The intergroup differences were found to be significant (Table 3 and Table 4). Gingival recession levels had also improved, however, the difference was not statistically significant (Table 5).

Evaluation of the hard tissue findings indicated that all treatment modalities resulted in bone gain at 9 months in both groups (Table 6).

TABLE 1**COMPARISON OF PLAQUE SCORE BETWEEN THE GROUPS AT DIFFERENT TIME INTERVALS**

Plaque Score	Group	Mean	StdDev	SE Of Mean	Kruskal-Wallis Chi-Sq	P-Value	Sig Diff Between
Baseline	Group 1	1.62	0.52	0.17	3.794	0.150	
	Group 2	1.73	0.30	0.09			
	Group 3	1.40	0.39	0.12			
3Months	Group 1	1.45	0.55	0.17	1.252	0.535	
	Group 2	1.30	0.26	0.08			
	Group 3	1.22	0.29	0.09			
6Months	Group 1	1.35	0.34	0.11	3.173	0.205	
	Group 2	1.35	0.41	0.13			
	Group 3	1.11	0.14	0.05			
9Months	Group 1	1.50	0.33	0.11	11.375	0.122	
	Group 2	0.95	0.28	0.09			
	Group 3	1.08	0.30	0.10			

*denotes significant difference (P value <0.05)

TABLE 2 **COMPARISON OF GINGIVAL INDEX (GI) SCORE BETWEEN THE GROUPS AT DIFFERENT TIME INTERVALS**

GI Score	Group	Mean	StdDev	SE Of Mean	Kruskal-Wallis Chi-Sq	P-Value	Sig Diff Between
Baseline	Group 1	1.52	0.48	0.15	4.211	0.122	
	Group 2	1.65	0.24	0.08			
	Group 3	1.26	0.45	0.14			
3Months	Group 1	1.15	0.47	0.15	2.940	0.230	
	Group 2	1.25	0.26	0.08			
	Group 3	0.98	0.33	0.10			
6Months	Group 1	1.03	0.34	0.11	4.504	0.105	
	Group 2	1.25	0.26	0.08			
	Group 3	1.05	0.24	0.07			
9Months	Group 1	1.55	0.37	0.12	12.352	0.102	
	Group 2	1.15	0.24	0.08			
	Group 3	1.00	0.24	0.07			

*denotes significant difference (P value <0.05)

TABLE 3**COMPARISON OF PROBING POCKET DEPTH (PPD) BETWEEN THE GROUPS AT DIFFERENT TIME INTERVALS**

PPD	Group	Mean	StdDev	SE Of Mean	Kruskal-Wallis Chi-Sq	P-Value	Sig Diff Between
Baseline	Group 1	9.85	1.40	0.44	0.938	0.626	
	Group 2	10.30	1.06	0.33			
	Group 3	10.50	1.08	0.34			
3 Months	Group 1	6.25	1.38	0.44	10.414	0.005*	1 vs 3
	Group 2	5.55	0.80	0.25			2 vs 3
	Group 3	4.60	0.52	0.16			
6 Months	Group 1	5.00	1.22	0.39	13.237	0.001*	1 vs 2
	Group 2	5.35	0.61	0.19			1 vs 3
	Group 3	4.25	0.35	0.11			
9 Months	Group 1	4.50	0.71	0.22	14.146	0.001*	1 vs 3
	Group 2	4.25	0.50	0.16			2 vs 3
	Group 3	4.00	0.34	0.11			

*denotes significant difference (P value <0.05)

TABLE 4**COMPARISON OF CLINICAL ATTACHMENT LEVEL (CAL) BETWEEN THE GROUPS AT DIFFERENT TIME INTERVALS**

CAL	Group	Mean	StdDev	SE Of Mean	Kruskal-Wallis Chi-Sq	P-Value	Sig Diff Between
Baseline	Group 1	8.70	1.32	0.42	1.054	0.590	
	Group 2	8.45	1.17	0.37			
	Group 3	9.20	1.42	0.45			
3Months	Group 1	5.35	1.25	0.39	11.832	0.003*	1 vs 2
	Group 2	4.30	1.06	0.33			1 vs 3
	Group 3	4.00	0.90	0.28			2 vs 3
6Months	Group 1	4.05	0.83	0.26	16.680	<0.001*	1 vs 2
	Group 2	4.15	0.55	0.17			1 vs 3
	Group 3	3.75	0.46	0.15			2 vs 3
9Months	Group 1	3.55	1.02	0.32	6.122	0.047*	1 vs 3
	Group 2	3.35	0.85	0.27			2 vs 3
	Group 3	3.25	0.91	0.29			

*denotes significant difference (P value <0.05)

TABLE 5 **COMPARISON OF GINGIVAL RECESSION (GR) BETWEEN THE GROUPS AT DIFFERENT TIME INTERVALS**

GR	Group	Mean	StdDev	SE Of Mean	Kruskal-Wallis Chi-Sq	P-Value
Baseline	Group 1	0.20	0.42	0.13	0.680	0.712
	Group 2	0.05	0.16	0.05		
	Group 3	0.10	0.32	0.10		
3Months	Group 1	0.15	0.34	0.11	0.562	0.755
	Group 2	0.05	0.16	0.05		
	Group 3	0.10	0.32	0.10		
6Months	Group 1	0.10	0.21	0.07	0.558	0.757
	Group 2	0.05	0.16	0.05		
	Group 3	0.05	0.16	0.05		
9Months	Group 1	0.10	0.21	0.07	2.148	0.342
	Group 2	0.00	0.00	0.00		
	Group 3	0.05	0.16	0.05		

TABLE 6**COMPARISON OF DEPTH OF DEFECT BETWEEN THE GROUPS AT DIFFERENT TIME INTERVALS**

BL	Group	Mean	StdDev	SE Of Mean	Kruskal-Wallis Chi-Sq	P-Value	Sig Diff Between
Baseline	Group 1	7.90	1.15	0.36	5.528	0.063	
	Group 2	8.00	1.33	0.42			
	Group 3	9.00	1.18	0.37			
3Months	Group 1	5.55	1.06	0.33	9.457	0.009*	1 vs 2
	Group 2	4.75	0.84	0.27			1 vs 3
	Group 3	4.60	0.60	0.19			
6Months	Group 1	5.35	1.16	0.37	19.667	<0.001*	1 vs 2
	Group 2	4.45	0.42	0.13			1 vs 3
	Group 3	4.25	0.44	0.14			2 vs 3
9Months	Group 1	4.75	1.00	0.32	19.628	<0.001*	1 vs 2
	Group 2	4.20	0.44	0.14			1 vs 3
	Group 3	4.15	0.41	0.13			

*denotes significant difference (P value <0.05)

DISCUSSION

The results of the present study show that treatment of intrabony defects with PerioGlas® alone, PRP/ PerioGlas® or PPP/ PerioGlas® leads to significant PPD reduction, attachment, and radiographic bone gain compared to baseline values. Statistically significant differences in all of the investigated parameters were found between the treatments. PRP is a new application of tissue engineering and acts as a storage vehicle for growth factors, especially PDGF and TGF- β, both of which influence bone regeneration (Tozum et al, 2003). PRF is centrifuged blood without any addition and it has the characteristic of polymerizing naturally and slowly during centrifugation. The slow polymerization mode confers to the PRF membrane a particularly favourable physiologic architecture to support the healing process (Dohan et al, 2006b). It has also been shown that a combination of PRF and other bone graft materials have a superior effect in reducing pocket depth, improving clinical attachment levels and promoting defect fill (Lekovic et al, 2012). In this study, bioactive glass has been added to PRP and PRF to assess the regenerative potential of the material.

In a study by Lindhe et al, it was shown that, surgical procedures would induce loss of attachment if done in pockets shallower than 4.2mm (Lindhe et al, 1982). Hence, probing depth greater than 5mm was considered for the study.

Laurell et al, in his study, has shown that, to benefit from regenerative procedures, depth of defect should be at least 3-4 mm (Laurell et al,1998).

The coin toss method was used for randomization of the subjects to receive the various treatment modalities. This was in accordance with previous studies (Sharma et al, 2006b).In an article by Lachin J M, it has been said that a method of complete randomization like the coin toss method eliminates the potential for selection bias (Lachin,1988).

Probing Pocket Depth (PPD), Clinical Attachment Level (CAL) and Gingival Recession (GR) were assessed using a UNC 15 probe positioned along the grooves on a customized acrylic stent which was fabricated for each patient for providing a reproducible insertion axis for the probe. Similar technique has been adopted in other studies (Sharma,2011; Subbaiah,2011). Preoperative and postoperative comparability of probing measurements that do not use this standardized method may be open to question (Carranza,2006).

The depth of the angular bone loss was assessed to the closest 0.5 mm on the intraoral perapical radiograph taken using the paralleling cone technique with the radiographic grid in position which allowed for standardization. Other studies have also used this technique for assessment of bone defect and bone fill (Subbaiah,2011; Cardaropoli, 2002).

The Platelet Rich Plasma (PRP) preparation has been described by Tozum et al (2003). As a deviation from the original procedure, in this study, calcium gluconate was used as gelling agent instead of calcium chloride and bovine thrombin. The use of bovine thrombin has been associated with the development of antibodies to human clotting factors V, XI, and thrombin, resulting in a risk of potentially life-threatening coagulopathies (Smith et al, 2007). Studies by Silva et al (2012), Batista et al (2011) have used calcium gluconate as the gelling agent and have found it to be an excellent alternative to the use of calcium chloride and bovine thrombin. The PRF was prepared following the protocol developed by Choukroun et al(2006).This technique has been considered the most ideal for PRF preparation for treatment of periodontal endosseous defects.

The oral hygiene was maintained satisfactorily in all the subjects till the end of the study period, suggesting that the regular reinforcement of the oral hygiene instructions was beneficial. The Hawthorne effect might have played an important role with regard to oral hygiene maintenance. Hawthorne effect is a form of reactivity whereby subjects improve or modify an aspect of their behavior, which is being experimentally measured, in response to the fact that they know that they are being studied (McCarney, 2007).

PerioGlas® alone, PRP/ PerioGlas® and PRF/ PerioGlas® combination have shown significant improvement in all the parameters except in terms of gingival recession. The bioactive glass synthetic bone graft particles (PerioGlas®) is an element-enriched dental bone-grafting system that actively supports bone regeneration. Filling the site with PerioGlas® initiates a unique chemical reaction known as "osteostimulation," which encourages new bone activity while its scaffolding network supports the new growth. PerioGlas® is uniquely versatile and effective in healthy bone regeneration and ideal for a wide range of osseous conditions (Froum et al, 1998; Subbaiah et al, 2011)

Platelet-rich plasma (PRP) is an autologous source of PDGF and TGF-β that is obtained by sequestering and concentrating platelets by gradient density centrifugation. It is a concentrated suspension of growth factors that has been demonstrated to induce healing and regeneration of tissues, including those in the periodontal area (Tozum et al, 2003). However, there are other studies that show that PRP does not have any additional effects over the other graft materials in the treatment of endossous defects (Ozdemir et al, 2012; Lafzi et al, 2013).

PRF has the characteristic of polymerizing naturally and slowly during centrifugation. The slow polymerization mode confers to the PRF membrane a particularly favourable physiologic architecture to support the healing process (Dohan et al, 2006b).PRF has been shown to act as suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for bone tissue engineering applications (Sharma et al, 2011a). PRF also induces proliferation of various cells in vitro with strongest induction effect on osteoblasts (Sharma et al, 2011a).

The comparison between the use of Platelet-Rich plasma and Platelet- Rich Fibrin has been done in a study which showed that there was similar PD reduction, CAL gain, and bone fill at sites treated with PRF or PRP with conventional open-flap debridement. Because PRF is less time consuming and less technique sensitive, it would seem a better treatment option than PRP (Pradeep et al, 2012).

The Platelet-Rich Fibrin and Platelet-Rich Plasma appear to have nearly comparable effects, with Platelet-rich Fibrin displaying slightly superior effect in comparison to Platelet Rich Plasma. Platelet-Rich Fibrin and Platelet-Rich Plasma have shown a better result in comparison to the bioactive glass synthetic bone graft particles alone. There are no studies in literature comparing the combination of graft materials used in this study. Hence a direct reference and comparison with the existing literature was not possible.

The slight superiority of PRF over PRP may be explained on the basis of the difference in their fibrin matrix. One of the main difference between fibrin adhesives, PRP and PRF is attributable to the gelling mode. In the formation of PRP, there is a constitution of a rigid network, not very favourable for cytokine enmeshment and cellular migration.

However, in PRF gel, the fibrin fibrillae form connected junctions that allow the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration. Moreover, this 3-dimensional organization will give great elasticity to the fibrin matrix and a flexible, elastic, and very strong PRF membrane is formed (Dohan et al,2006b).

CONCLUSION

Treatment with bioactive glass and autologous PRF or PRP stimulates a significant increase in the PD reduction, CAL gain, and bone fill. The use of PRP and PRF appear to be a better option than alloplastic materials, as PRF and PRP are autologous preparations from patient's own blood. The use of PRF and PRP also decreases the cost of the regeneration therapy. Placement of PRF and PRP are not technique sensitive and hence there is no learning curve to the use of these autologous platelet concentrates.

PRF appears to have a slight advantage over PRP in its efficacy in the treatment of periodontal endosseous defects. However, long-term, randomized, controlled clinical trial and histomorphometric studies will be needed to arrive at a definitive conclusion.

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