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Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: a controlled clinical trial

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Abstract

Aim: Platelet-rich fibrin (PRF) may be considered as a second-generation platelet concentrate widely used to accelerate soft and hard tissue healing because of presence of many growth factors. The present study aimed to investigate the clinical and radiological effectiveness of autologous PRF in the treatment of intra-bony defects of chronic periodontitis patients.

Material and Methods: Thirty-two intra-bony defects (one site/patient) were treated either with autologous PRF or a conventional open flap debridement alone. Clinical parameters such as plaque index (PI), sulcus bleeding index (SBI), probing depth (PD), clinical attachment level (CAL) and gingival marginal level (GML) were recorded at baseline and 9 months post-operatively. In both the groups, by using the image analysis software intra-bony defect fill was calculated on standardized radiographs (from the baseline and 9 months).

Results: For all clinical and radiographic parameters test group was performed better than control group, and the difference was found to be statistically significant. Furthermore, images analysis revealed significantly greater bone fill in the test group compared with control (46.92% *versus* 28.66 %). Mean PD reduction ($4.56 \pm 0.37 > 3.56 \pm 0.27$) and CAL gain ($3.69 \pm 0.44 > 2.13 \pm 0.43$) in test group was found to be more compared with that of control group. In the test group, PD of >4 mm has highest percentage of PD reduction (68.9%) and CAL gain (61.6%). On frequency distribution analysis, there was no more difference for PD reduction in both the groups but CAL gain was much more in the test group than the control group.

Conclusions: Within the limit of the present study, there was greater reduction in PD, more CAL gain and greater intra-bony defect fill at sites treated with PRF than the open flap debridement alone.

Key words: intra-bony defects; periodontal regeneration; periodontitis; platelet-rich fibrin

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Regeneration has been defined as the reproduction or reconstitution of a lost or injured part to restore the architecture and function of the periodontium (American Academy of Periodontology 1992). When periodontal disease causes a loss of the attachment apparatus, optimal care seeks to regenerate the periodontium to its pre-disease state. The primary goal of periodontal treatment is the maintenance of the natural dentition in health and comfortable function (American Academy of Periodontology 1992). To be considered a regenerative modality, a material or technique must histologically demonstrate that bone, cementum and a functional periodontal ligament (a new attachment apparatus) can be formed on a previously diseased root surface (Zander et al. 1976).

Since 1923 [from Hegedus (1923) time], a number of techniques and various grafting materials have been in use for periodontal tissue regeneration (Robinson 1969, Schallhorn & Hiatt 1972, Carraro et al. 1976, Blumenthal et al. 1986,

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Bowers et al. 1986, Rosenberg & Cutler 1994). However, among the graft materials only autogenous bone of extra-oral or intra-oral sources (Dragoo & Sullivan 1973, Hiatt et al. 1978) and demineralized freeze-dried bone allograft (Bowers et al. 1989) have human histological evidence to include them as regenerative materials (Cortellini & Bowers 1995). More recently, the use of platelet-rich plasma (PRP) (Anitua et al. 2004), enamel matrix derivative (EMD) (Sculean et al. 1999), recombinant human bone morphogenetic proteins (BMP)-2 (Ishikawa et al. 1994), growth factors like platelet-derived growth factor (PDGF) and insulin-like growth factor-1 (IGF-1) (Lynch et al. 1989) and recombinant human basic fibroblast growth factor (bFGF) (Murakami et al. 2003) have been proposed as a source for periodontal regeneration. PRP is an autologous concentration of platelets in a small volume of plasma with high concentration of the fundamental protein growth factors like PDGF, transforming growth factor (TGF-1 and -2), vascular endothelial growth factor (VEGF), IGF-1 and -2, bFGF and epithelial growth factor (EGF) secreted by platelets to initiate wound healing (Marx et al. 1998, Babbush et al. 2003).

Platelet-rich fibrin (PRF) may be considered as a second-generation platelet concentrate, using simplified protocol (Dohan et al. 2006, Dohan Ehrenfest et al. 2009a, b). Carroll et al. (2005) in vitro study demonstrated that the viable platelets in PRF released six growth factors like, PDGF, VEGF, TGF, IGF, EGF and bFGF in about the same concentration for the 7-day duration of their study. More recently, a canine study in mongrel dogs by Simon et al. (2009) determined the effect of PRF in extraction sites treated with PRF matrix with or without membrane and found PRF exhibit enhanced healing compared with sites treated with non-viable materials and showed that healing was more rapid in the PRF and PRF with membrane treated sites and found by 3 weeks those sockets had osseous fill. Again its beneficial effect in various surgical procedures like, sinus floor augmentation during implant placement (Mazor et al. 2009); in multiple gingival recessions with coronally displaced flap (Del Corso et al. 2009) and in facial plastic surgical procedures (Sclafani 2009) has been evaluated. The effect of PRF on human primary cultures of gingival fibroblasts, dermal pre-keratinocytes, pre-adipocytes and maxillofacial osteoblasts was evaluated in vitro (Dohan et al. 2009a, b). He demonstrated that, PRF induced a significant and continuous stimulation and proliferation of all cell types. The effect was dose dependent during all the experiment with osteoblasts, but only on day 14 with fibroblasts. Moreover, PRF induced a strong differentiation in the osteoblasts. The analysis of osteoblasts cultures in differentiation conditions with PRF using light and scanning electron microscopy revealed a starting mineralization process in the PRF membrane (Dohan et al. 2009a, b). Again, there are various advantages PRF over PRP. It requires less chair side time (12 min.) for preparation than PRP (Dohan et al. 2006); no need of addition of bovine thrombin-like PRP; long term effect of growth factors than PRP (Carroll et al. 2005) and it can be easily formulated into the membrane like guided tissue regeneration (GTR) membrane. Because of the above-mentioned advantages over the PRP (which is a first-generation platelet concentrate, as it was developed first), PRF is considered as a second-generation platelet concentrate (Dohan et al. 2009a, b).

Keeping the above facts in mind and prolong release of various growth factors, it would be expected that PRF treatment of an intra-bony defect (IBD) may results in enhanced wound healing and periodontal regeneration compared with those sites treated with conventional open flap debridement. To test this hypothesis, the present study was carried out as a single-centre controlled clinical trial to investigate the clinical and radiological (bone fill) effectiveness of autologous PRF in the treatment of IBDs of chronic periodontitis patients.

Material and Methods

Patient selection

Forty systemically healthy subjects [22 male and 18 female, age range: 25–45 (31.12 ± 2.06) years] undergoing periodontal therapy at the Department of Periodontics, Government Dental College and Research Institute (from April 2009 to January 2010) were selected for the study. Intra-oral periapical radiographs (IOPAs) were taken to confirm the presence of suitable IBDs for the selection of subjects.

The inclusion criteria were the presence of inter-proximal IBDs $\geqslant 3$ mm deep (distance between alveolar crest and base of the defect on IOPA) along with an inter-proximal probing depth (PD) $\geqslant 5$ mm following phase I therapy (scaling and root planing, SRP) in vital, asymptomatic first and second mandibular molars without furcation involvement. Patients with present or past systemic illness that known to affect the outcomes of periodontal therapy, insufficient platelet count ($<200{,}000/mm^3$), immune compromised patients, pregnancy/lactation and smoking (any other tobacco products), were excluded from the study. Patients taking medications that may interfere with wound healing, those allergic to other medication and having unacceptable oral hygiene [if plaque index (PI) $\geqslant 3$] after the re-evaluation of phase I therapy were excluded from the study.

Written informed consent was obtained from those who were agreed to participate. Ethical clearance (No: GDCB/143/12 March 2009) was received from the Institutional Committee of Government Dental College and Research Institute, Bangalore.

Pre-surgical therapy

Before the surgery, each patient was given careful instructions on proper oral hygiene measures. Full-mouth supra and subgingival SRP procedures (with ultrasonic instrument, EMS V-Dent, Guangdong, China) were performed under local anaesthesia.

Six to 8 weeks following phase I therapy, periodontal evaluation was performed to confirm the suitability of the sites for this study. The selected sites were divided randomly (coin toss) into the control and test groups. The control group consisted of the sites treated with conventional flap surgery, whereas the test group sites were treated with conventional flap surgery with autologous PRF. In both the groups Kirkland's modified flap operation (Kirkland 1931) procedure was performed followed by suturing and periodontal dressing for 7–14 days. In the control and test group one site/subject was treated.

Clinical parameters recorded before the surgical procedures included PD [measured from the gingival margin to the base of the pocket (tip of the probe in the pocket)], clinical attachment level (CAL), measured from the cemento-enamel junction (CEJ) to the base of the pocket (tip of the probe in the pocket) and gingival marginal level (GML, measured from the CEJ to the level of the gingival margin), using customized acrylic stents with grooves to ensure a reproducible placement of

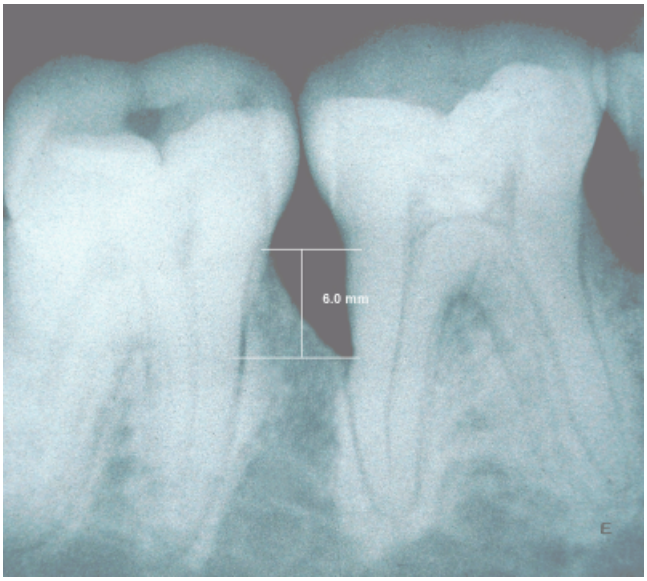

Fig. 1. Baseline radiograph showing IBD = 6.0 mm with linear measurement by Scion image analyzer.

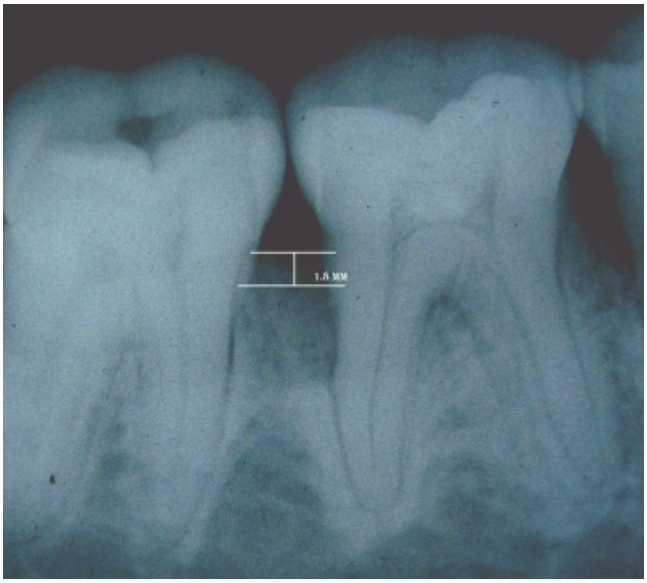

Fig. 2. Radiograph after 9 month showing IBD = 1.8 mm with linear measurement by Scion image analyzer.

the University of North Carolina no. 15 (UNC-15, HuFriedy, Chicago, IL, USA) periodontal probe. Site-specific PI (Silness & Loe 1964) and sulcus bleeding index (SBI) (Muhlemann & Son 1971) were also measured.

Radiographic evaluation of IBDs

IBD was evaluated at baseline and after 9 months (Figs. 1 and 2). The radiographic IBD depth (vertical distance from the crest of the alveolar bone to the base of the defect) was measured by means of computer aided program (Scion image Corporation, Fedrick, MD, USA) used in our previous study (Pradeep & Thorat 2010). Individually customized bite blocks and parallel angle technique was used to obtain standardized radiographs. A review of all the radiographs was performed in a single reference center by a blind evaluator. For evaluation, radiographs were scanned at 800 dpi with a scanner (HP Scanjet 3c/I, Hewlett Packard, Palo Alto, CA, USA).

PRF preparation

The PRF was produced according to the protocol – Process protocol, Nice, France; developed by Choukroun et al. (2001). PRF was prepared without biochemical manipulation of blood. On the day of surgery, 10 ml of blood was drawn from each patient by venipuncture of the antecubital vein. Blood was collected in a sterile glass test tube (10 ml) without any anti-coagulant. Immediately test tube was centrifuged using a refrigerated centrifugal machine at 400 *g* for 12 min. Because of differential densities, it resulted in the separation of three basic fractions: a base of red blood cells at the bottom, acellular plasma on the surface, and finally a PRF clot between the two. A total of 2–3 ml of the top layer was pipetted out with the sterile dropper; the middle layer (PRF) was removed and placed in a sterile dappen dish.

Surgical procedure

Following administration of local anaesthesia, buccal and lingual sulcular incisions were made and the mucoperiosteal flaps were elevated. Care was taken to preserve as much inter-proximal soft tissue as possible. Meticulous defect debridement and root planing was carried out with the use of curettes. No osseous recontouring was performed.

PRF gel was prepared according to the method described above. One part of PRF was placed in the IBD and the other part was used to prepare the membrane which was used to cover the defect as a GTR membrane. To avoid the displacement of PRF, a suture was passed through the buccal and lingual flap before the placement of PRF. The mucoperiosteal flaps were repositioned and secured in place using 3-0 non-absorbable black silk surgical suture (Ethicon, Johnson & Johnson, Somerville, NJ, USA). The Modified vertical mattress and interrupted sutures were placed. The surgical area was protected and covered with periodontal dressing (Coe-Pak, GC America, Alsip, IL, USA). Later, suitable antibiotics (amoxicillin, 500 mg, every 8 h) and 0.2% chlorhexidine digluconate (CHX) rinse (twice daily for 2 weeks) was prescribed. Surgical procedures in test and control groups were differed for PRF treatment only.

Single surgeon (M. T.) performed all surgeries. An examiner (A. R. P.) other than the operator performed all clinical measurements without knowledge of the treatment groups.

Post-operative care

Periodontal dressing and sutures were removed 2 weeks post-operatively.

Table 1. Sulcus bleeding index (SBI) and plaque index (PI) at Baseline and 9 months

	Baseline, *n* (%)			9 months, *n* (%)		
	control	test		control	test	
SBI						
0	0	0		10 (62.5)	11(68.8)	
1	9 (56.2)	10 (62.5)		5 (31.3)	5 (31.3)	
2	7 (43.8)	6 (37.5)		1 (6.2)	0	
p-value	< 0.001*		0.05*	< 0.001*		
PI						
0	0	0		11 (68.8)	13 (81.3)	
1	12 (75.0)	12 (75.0)		5 (31.3)	3 (18.7)	
2	4 (25.0)	4 (25.0)		0	0	
p-value	0.003*		0.01*	<0.001*		

*Statistically significant at $p<0.05$.

Table 2. Comparison of two groups and baseline and 9 months in both the groups with respect to PD (in mm)

Group	Mean ± SD			%change
	baseline	9 months	baseline –9 months	
Group I	6.75 ± 1.69	3.19 ± 1.52	3.56 ± 1.09	52.78*†
Group II	7.88 ± 2.19	3.19 ± 1.05	4.69 ± 1.45	59.52*†
F-value	2.6471	3.2301		
p-value	0.1142‡	0.0827§		

*$p<0.01$.
†Paired *t*-test.
‡One-way ANOVA.
§Analysis of covariance (ANCOVA) by taking baseline values as a covariate.

Table 3. Comparison of two groups and baseline and 9 months in both the groups with respect to clinical attachment level (CAL) (mm)

Group	Mean ± SD			% change
	baseline	9 months	baseline –9 months	
Group I	6.50 ± 1.75	4.38 ± 2.16	2.13 ± 1.71	32.69*†
Group II	7.69 ± 1.82	3.56 ± 2.06	4.13 ± 1.63	53.66*†
F-value	3.5462	7.8416		
p-value	0.0694‡	0.0090*§		

*$p<0.01$.
†Paired t-test.
‡One-way ANOVA.
§Analysis of covariance (ANCOVA) by taking baseline values as a covariate.

Surgical wounds were gently cleansed with 0.2% CHX on a cotton swab. Thereafter, gentle brushing with a soft toothbrush was recommended. At 8 weeks post-operatively, each patient was reinstructed about proper oral hygiene measures. Patients were examined weekly for 1 month after surgery and then at 3, 6 and 9 months. No subgingival instrumentation was attempted at any of these appointments. Post-operative care included reinforcement of oral hygiene and mechanical plaque control whenever necessary.

Post-surgical measurements

PI, SBI, PD, CAL and GML were recorded 9 months after the initial surgery. Soft and hard tissue evaluation was performed. Soft tissue measurements were repeated with previously used acrylic stents. A second IOPA (after 9 months) of the same treated sites was taken, and IBD measurements were performed from the baseline and 9-month radiographs.

Statistical analysis

The results were averaged (mean ± SD) for each clinical and radiographic parameter at baseline and 9 months. The difference between each pair of measurements was calculated (baseline – 9 months). The paired *t*-test was applied to assess the statistical significance between time points within each group for the clinical and radiographic parameters. One-way analysis of variance (ANOVA) was used to test the difference between the groups. SBI and PI were expressed as absolute and relative counts. Comparison of indices between test and control groups was carried out using the Mann–Whitney test. The data were analysed using statistical software (SPSS version 10.5, SPSS, Chicago, IL, USA). A small scale study called a pilot study was conducted 6 months before, based on the pilot study results, the standard deviation of a parameter (GI or PI or CAL) is in first groups is 0.40 and second group is 0.42, the mean difference was calculated between the two groups was 0.45. Using this information, the sample size was calculated under 5% error (to tolerate) and power of the test was about 85% and considering two-sided test. The required sample size was found to 15 in each group.

The mean intra-examiner standard deviation of differences in repeated PD measurements and CAL measurements obtained using single passes of measurements with a conventional probe (correlation coefficients between duplicate measurements; $r = 0.730$).

Results

Out of 40, 32 patients (control group 30.3 ± 4.3 years, nine males, seven females; test group 31.12 ± 4.9 years, 11 males, five females) were completed the study. Four patients were not reported to the clinic as they either refused to participate due to reasons unrelated to this study or not meet the inclusion criteria. After interventional therapy, four more patients (two from test group and two from control group) were either not followed up or discontinued the treatment. Thirty-two treatment sites (one site/subject) were evaluated and analysed for clinical (PD, CAL and GML) and radiological

Table 4. Comparison of two groups and baseline and 9 months in both the groups with respect to gingival recession (GML)

Group	Mean ± SD			%change
	baseline	9 months	baseline – 9 months	
Group I	0.19 ± 0.40	1.50 ± 1.03	− 1.31 ± 1.01	− 700.00*†
Group II	0.50 ± 0.82	0.81 ± 0.75	− 0.31 ± 0.95	− 62.50*†
F-value	1.8844‡	5.8548		
p-value	0.1800	0.0220*§		

*$p < 0.01$.
†Wilcoxon's matched paired test by ranks *t*-test.
‡One-way ANOVA.
§Analysis of covariance (ANCOVA) by taking baseline values as a covariate.

Table 5. Intra-bony defect (IBD) depth in mm

Subject no.	Baseline		9 months	
	control	test	control	test
1	3.4*	4.2†	2.5	2.1
2	5.1*	6.2*	4.0	2.7
3	6.5*	4.7*	4.9	2.7
4	4.8*	3.4*	3.6	1.9
5	3.7†	6.2*	2.8	3.7
6	3.8*	5.6*	2.5	3.6
7	3.2*	3.2*	2.3	1.9
8	4.5†	4.5†	3.5	2.3
9	3.3*	4.6*	2.3	2.8
10	6.0*	4.6*	3.2	2.0
11	4.1†	4.0*	3.2	2.1
12	5.1*	6.0*	3.5	1.8
13	4.4†	4.5†	3.4	2.6
14	3.4*	3.0*	1.8	1.8
15	3.7*	3.3†	1.5	1.7
16	5.8†	4.2*	4.5	1.9
Mean ± SD	4.41 ± 1.02	4.52 ± 1.11	3.08 ± 0.92	2.35 ± 0.52

*Three-wall defect.
†Two-wall defect.

Table 6. Comparison of mean and percentage reduction of IBD (mm) from baseline to 9 months

Groups	Mean ± SD	Mean ± SD reduction	Percentage of IBD reduction	*t*-value	*F*-value	*p*-value
Test ($n = 16$)						
Baseline	4.52 ± 1.11	2.12 ± 0.69	46.92	12.12	13.31	< 0.001*
9 months	2.39 ± 0.71					
Control (n = 16)						
Baseline	4.40 ± 1.04	1.24 ± 0.69	28.66	7.11		< 0.001*
9 months	3.08 ± 0.92					

F-value: analysis of variance.
*Statistically significant at $p < 0.001$.

parameters (IBD fill) at baseline and 9 months in 32 treatment sites. The mean PD and CAL at baseline and 9 month in control and test group was 6.75 ± 1.69, 6.50 ± 1.75 and 3.19 ± 1.52, 4.38 ± 2.16 mm, respectively, and was found to be significant ($p < 0.01$) at 9 months. When IBD at baseline (4.40 ± 1.04) and test group (4.52 ± 1.11) was compared, it was found to be non-significant at baseline ($p > 0.05$) and was significant at 9-month comparison ($p < 0.05$) (Table 6).

Healing of all the control and test sites was uneventful and not a single case of post-operative infection was observed. Reductions in the SBI and PI were observed in both groups at 9 months post-operatively and are statistically not significant. The differences between two groups were statistically insignificant when compared at baseline and 9 months ($p > 0.05$) and was found to be significant when compared with baseline *versus* 9 months ($p < 0.05$) (Table 1).

Changes in the clinical parameters are reported in Tables 2–4. When the mean difference was found to be significant ($p < 0.01$) when compared in both the groups at baseline and 9 months (Table 2). CAL gain was found to be significantly greater in the test site (3.56 ± 2.06) compared with control site (2.13 ± 0.43) at 9-month visit (Table 3). When GML were compared between the groups, it was significant ($p < 0.05$) with less marginal tissue recession in test group (0.81 ± 0.75) compared with control site (1.50 ± 1.03) after 9 months of examination (Table 4).

Changes in the radiographic parameters are reported in Table 6. Test sites presented with a mean defect fill (distance from alveolar crest to the base of the defect at baseline and 9 months post-operatively) of 2.12 ± 0.69 mm and the percentage defect fill of 46.92%, which was higher than the control sites (1.24 ± 0.69; 28.66%). This difference was significantly greater in the test group than in the control group ($p < 0.05$). Out of 16 treated IBDs, 11 (68.75%) defects were three-walled and five (31.25%) were two-walled in the control group, and in the test group 12 (75.0%) defects were three-walled and four (25.0%) were two-walled. On observation, the data represented in Table 5 shows that compared with two-walled the three-walled IBD had more defect fill.

Frequency distribution table (Table 6) shows more PD reduction, less residual PD and more CAL gain for the PRF treated group than the control group (Table 7).

Discussion

The present study was aimed to evaluate the clinical effectiveness of PRF in the treatment of IBD in chronic periodontitis patients. In total, 32 subjects (16 subject per group, one site/subject) was treated either with the conventional periodontal flap surgery alone or conventional flap surgery with autologous PRF. To the best of our knowledge, till date there is no published data on the use of PRF in the IBD of periodontitis patients.

Reconstructive dental surgeons are constantly looking for an ''edge'' that

Table 7. Frequency distribution tables for probing depth (PD) reduction, residual PD and clinical attachment level (CAL) gain

Parameter	In mm	Frequency	%	Mean ± SD
PD reduction				
Control group	⩽3	7	43.9	3.56 ± 0.27
	4–6	9	56.1	
Test group	⩽3	5	31.3	4.56 ± 0.37
	4–6	10	62.6	
	⩾7	1	6.3	
CAL gain				
Control group	⩽3	11	68.9	2.13 ± 0.43
	4–6	5	31.3	
Test group	⩽3	6	37.6	3.69 ± 0.44
	4–6	8	49.1	
	⩾7	2	12.5	
Reduced PD				
Control group	⩽3	11	68.8	3.19 ± 1.51
	4–6	5	31.3	
Test group	⩽3	10	62.6	3.19 ± 1.04
	4–6	6	37.5	

jumps starts the healing process to maximize predictability as well as the volume of regenerated bone. Many growth factors like BMP-2, bFGF, recombinant PDGF-BB and PRP have been studied in animals and are in use in the clinical practice to treat the suprabony or IBDs due to periodontitis.

Reduction in PD, IBD and gain in CAL are the major clinical outcomes measured to determine the success of any periodontal treatment. In the present study, a significant reduction in PD and CAL gain were found in both groups when compared with baseline and 9 months. However, there was more PD reduction (4.56 ± 0.37) and CAL gain (3.69 ± 0.44) in the PRF-treated group compared with the subjects treated with conventional periodontal flap surgery alone. The present study also reflects the percentage of IBD fill in the PRF group (46.92%) is higher than the conventionally treated subjects (28.66%), supporting the significance and advantage of various growth factors present in the PRF may accelerate the soft and hard tissue healing (Choukroun et al. 2001, Dohan et al. 2007). Also, Simon et al. (2009) observed that the healing was more rapid in the PRF and PRF with membrane treated sites with significant osseous fill in sockets by 3 weeks. In vitro study by He et al. (2009) on rat osteoblasts showed that cells treated with exudates of PRF collected at day 14 reached peak mineralization significantly more than both negative control and positive (PRP) control groups and concluded that PRF is superior to PRP, from the aspects of expression of alkaline phosphatase (ALP) and induction of mineralization, because of markedly released TGF-β1 and PDGF-AB. Again, by using light and scanning electron microscopy revealed that osteoblasts cultured in differentiation conditions with PRF showed a starting mineralization process in the PRF membrane itself (after 14 days) and PRF leucocytes seemed to proliferate and interact with osteoblasts (Dohan Ehrenfest et al. 2009a, b). Mazor et al. (2009) by radiologic and histologic analyses showed that the use of PRF during a simultaneous sinus lift and implantation stabilizes a high volume of natural regenerated bone in the subsinus cavity, up to the tip of the implants.

There are many advantages of using PRF, a second-generation platelet concentrate, over the PRP. First, PRF differs from other commercially available PRP systems in that it does not use bovine thrombin or other exogenous activators in the preparation process. The PRF preparation process creates a gel-like matrix that contains high concentrations of non-activated, functional, intact platelets, contained within a fibrin matrix, that release, a relatively constant concentration of growth factors over a period of 7 days (Carroll et al. 2005). Second, it can be squeezed to form a membrane and can be used as fibrin bandage serving as a matrix to accelerate the healing of wound edges (Gabling et al. 2009, Vence et al. 2009). Third, the chair side preparation of PRF is quite easy and fast and simplified processing minus artificial biochemical modification than PRP, which takes more time (Dohan et al. 2006). Fourth, this produces an inexpensive autologous fibrin membrane in approximately 1 min. and hence no cost for membrane and bone graft to the patients.

A recent 6-month study evaluated the use of PRF in the treatment of multiple gingival recessions with coronally advanced flap procedure and found the significant improvement during the early periodontal healing phase with a thick and stable final remodelled gingiva (Del Corso et al. 2009). In the present study, it has been found that there was less marginal tissue recession in test group compared with control sites after 9 months of examination (test site, 0.81 ± 0.75; control site, 1.56 ± 0.96). A recent in vivo dog study showed that, sites treated with PRF and demineralized freeze-dried bone allograft (DFDBA) healed slower than those with PRF alone or with a membrane, but faster than those grafted with DFDBA and a membrane (Simon et al. 2009). A 9 months human clinical trial using high dose (150 μg/ml) recombinant human PDGF/recombinant human IGF-1 revealed 2.08 mm of new bone and 43.2% defect fill, compared with 0.75 mm vertical bone height and 18.5% bone fill in controls (Howell et al. 1997). Study by Heijl et al. (1997) showed the mean radiographic bone fill was greater for the EMD treated defects that for the control sites (2.7 *versus* 0.7 mm, respectively) with mean PD (3.1 *versus* 2.3 mm, respectively) and mean CAL gain (2.2 *versus* 1.7 mm), respectively. The present study is also in accordance with respective to the defect fill, gain in CAL and reduction in PD when compared with that of control. However, long-term and large sample studies should be carried out to affirm the observations of our study.

Two recent reviews by Laurell et al. (1998) and Lang (2000), reported the weighted mean bone defect fill in the angular defect by open flap debridement is 1.1 and 1.5 mm, respectively. In the present study, the results of control group is in accordance with these recent reviews showing the mean angular defect fill of 1.24 ± 0.69 mm. Most of the defects treated in the present study are three wall and two wall. One also has to consider that the potential for bone fill may differ depending on the morphology of the angular bone defect. Most angular defects appear as combinations of one-, two- and three-wall defects and whereas the two- and three-wall component of an angular

bone defect may show great potential for bone fill during healing, the one-wall component will rarely demonstrate this type of healing.

Growth factors like recombinant PDGF-BB and allograft like DFDBA were found to be more effective than other xenografts, alloplasts and GTR in the periodontal regeneration. As, PRF is an autologous preparation from patients own blood like PRP, it decreases the cost of the regeneration therapy and also less time consuming, both for the surgeon and patient. Again placement of PRF does not require a skill and it is less technique sensitive than GTR and bone graft placement. However, long-term, randomized, controlled clinical trial will be needed to know its effect over the other treatment modalities.

Conclusions

Within the limit of this study, there was greater reduction in PD, and more CAL gain with significant IBD filled with PRF in IBDs. However, long-term and large sample studies should be carried out to affirm the observations of our study. Use of PRF in the periodontal regeneration procedures would be the cost effective and less technique sensitive treatment both for the patients and clinician.

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Clinical relevance

Scientific rationale for the study: This is the first clinical trial to study the clinical effectiveness of PRF in the treatment of IBDs in chronic periodontitis patients.

Principal findings and practical implications: Treatment of IBDs with autologous PRF placement in adjunct to conventional flap procedures showed that a significant improvement in the clinical parameters and significant bone fill same as the use of PDGF and EMD, however further studies are required.

Advantages of PRF: Not required biochemical modification like PRP, cost effective than other growth factors (PDGF, EMD), faster healing, and elimination of disadvantages involved in using barrier membranes, release of constant concentration of growth factors for prolonged period of time than PRP.