Original Research

Comparative Evaluation of Platelet-Rich Fibrin Biomaterial and Open Flap Debridement in the Treatment of Two and Three Wall Intrabony Defects

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Abstract:
Background: Platelet-rich concentrates are the most widely used regenerative biomaterials. Stimulation and acceleration of soft and hard tissue healing are due to local and continuous delivery of growth factors and proteins, mimicking the needs of the physiological wound healing and reparative tissue processes. This article aims to evaluate the clinical efficacy of open flap debridement (OFD) with or without platelet-rich fibrin (PRF) in the treatment of intrabony defects.

Materials and Methods: Twenty subjects with forty intrabony defects were treated with either autologous PRF with open-flap debridement (test, *n* = 20) or open-flap debridement alone (control, *n* = 20). Soft tissue parameters included: Plaque index, sulcus bleeding index, probing depth, relative attachment level and gingival marginal level (GML). The hard tissue parameters included-distances from: Cement enamel junction to the base of the defect (CEJ-BOD): Alveolar crest to the base of the defect (AC-BOD): And CEJ to AC. The parameters were recorded at baseline and at 9 months postoperatively calculated using standardized radiographs by image-analysis software.

Results: Statistically significant (0.005*) intragroup improvements were seen with all the hard and soft parameters in both test and control groups, except for GML. Statistically significant improvements were seen with the mean defect fill (CEJ-BOD and AC-BOD) ($P = 0.003^*$) when intergroup comparisons were made.

Conclusions: Adjunctive use of PRF with OFD significantly improves defect fill when compared to OFD alone. PRF has consistently been showing regenerative potential; it is simple, easy and inexpensive biomaterial compared with bone grafts.

Key Words: Intrabony defect, open flap debridement, periodontal grafts, platelet rich fibrin, reconstructive osseous surgery

Introduction

The definitive goal of regenerative periodontal therapy aims at the reconstruction/regeneration of the tooth supporting apparatus, which has been lost because of periodontitis or trauma.[1] Periodontal regeneration is defined as the complete restoration of lost tissues to their original architecture and function by recapitulating the crucial wound-healing events associated with their development.[2]

Among the broad range of available treatment options, only a few may be regarded as truly regenerative. A material or technique must histologically demonstrate that periodontal tissue regeneration (new attachment) is formed on a previously diseased/destroyed root surface, in order to be considered a regenerative modality.[3] In the last two decades, various biomaterials[4-9] have been developed and experimented for periodontal tissue regeneration based on their endogenous regenerative capacity, but there is no graft material that is considered as the gold standard.[10]

Platelet-rich fibrin (PRF), an autologous fibrin material as described by Choukroun *et al.* belongs to the second-generation platelet concentrate with cicatricial properties.[11] The preparation of this material as described by Choukroun is a simplified and an inexpensive (The preparation does not require any anticoagulants, bovine thrombin or any other gelling agent) procedure.[12] It consists of an intimate assembly of cytokines, glycanic chains, and structural glycoproteins enmeshed within a slow releasing fibrin network.[12] This leads to more efficient cell migration and proliferation. This unique structure may act as a carrier for cells that are

essential for tissue regeneration. After collection of venous blood in dry 10 mL tubes, it was centrifuged for 12 min at 2700 rpm (~400 g). As soon as the centrifugation process was complete, there are 3 layers that are seen: At the bottom layer red blood cells are seen, at the top layer acellular plasma also known as platelet-poor plasma was seen and between the two layers PRF was seen. After the centrifugation process was complete, it was observed that ~97% of platelets and 50% of leukocytes of the original blood volume were concentrated in the PRF.[13] Previous studies have shown a slow release of growth factors such as transforming growth factor β1, platelet-derived growth factor β, and vascular endothelial growth factor, especially during the first 7 days.[14,15] Zumstein *et al.* reported that this release gradually slowed and continued up to 28 days.[16] This implies that the membrane stimulates its environment for a significant time during the wound healing process. PRF can be used alone,[17] or in combination with different bone substitutes.

Beneficial effects of PRF have been studied in various procedures, such as facial plastic surgery,[18] a sinus-lift procedure as a sole osteoconductive filling material,[19] and multiple gingival recessions cases treated with a coronally advanced flap.[20] PRF has been shown to act as suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for applications in bone tissue engineering.[21]

It is very well-known that regeneration of tissues destroyed by periodontal disease cannot be achieved by conventional open flap debridement (OFD) alone.[22] Thus, the use of PRF adjunctive to OFD is justified. Our previous studies[4,10,23,24] have evaluated the effects of PRF in only three walled intrabony defects. However, the aim of this study is to evaluate the adjunctive effect of platelet-rich fibrin and OFD in the treatment of both two-and-three walled intrabony defects.

Materials and Methods
Twenty systemically healthy subjects were included in this 9 months follow-up, longitudinal interventional study, (10 males and 10 females; mean age: 30.5 years) carried out in the Department of Periodontology and Oral Implantology, Dr. D.Y. Patil Dental College and Hospital, Dr. D Y Patil Vidyapeeth, Pune, India. The research protocol was initially submitted to the Institutional Ethical Committee and Review Board. Written informed consent was obtained from all those who agreed to participate voluntarily in the study. Inclusion criteria included: Two and three-wall intrabony defects ≥3 mm (distance from alveolar crest to base of the defect [AC-BOD]), defect on an intraoral periapical radiograph [IOPA] along with a probing depth (PD) of ≥5 mm after Phase 1 therapy (scaling and root planning [SRP] in an asymptomatic tooth. Subjects with known systemic disease or on any medications known to interfere with the outcomes of periodontal therapy, or subjects

using tobacco in any form, or subjects who have undergone any periodontal therapy in the preceding 6 months, pregnant or lactating mothers, were excluded from the study. Patients who had unacceptable oral hygiene (plaque index [PI] of >1.5) after the reevaluation of Phase 1 therapy were also excluded from the study. In addition, furcation defects, nonvital teeth, external root resorption were also excluded.

Nonsurgical periodontal therapy (Phase 1 therapy)
At the initial visit, each patient underwent a full-mouth supra and subgingival SRP. In the first visit, all patients were given careful instructions regarding proper oral hygiene maintenance. Six-week post SRP, a periodontal evaluation was done to confirm the desired sites for the study.

Randomization
The selected sites were divided randomly (computer generated list) into control and test groups. The control group consisted of sites treated with OFD alone, whereas test-group sites were treated with OFD with autologous PRF. One operator (HA) performed all surgeries, whereas another investigator (SS) performed all clinical assessments, and the radiographic measurements were done by third investigator (RK).

Clinical and radiographic measurements
The clinical parameters recorded before surgical procedures included site-specific PI, sulcus bleeding index (mSBI), PD, relative attachment level (RAL) along with gingival marginal level (GML). They were recorded from the apical level of the pre-fabricated custom acrylic stents with grooves to ensure accurate placement of the University of North Carolina (UNC) no. 15 periodontal probe (UNC-15 periodontal probe, Hu-Freidy, Chicago, IL, USA). All IBD was evaluated at baseline and 9 months postoperatively. For the measurement of bone defect, distance from the crest of the alveolar bone to the base of the defect (AC-BOD) was considered. Paralleling angle technique with 1 mm × 1 mm grid was used to obtain standardized radiographs. For assessment, radiographs were scanned with a scanner (Epson Perfection V700, Epson, Pune, Maharashtra, India) of 6400 DPI by an evaluator (RK) who was blinded to surgical procedure performed in subjects. The radiographic IBD depth was measured by computer aided software program (Epson Perfection V700, Epson, Bangalore, India) as used in our previous studies.[23-25]

PRF preparation
The PRF was prepared following the protocol developed by Choukroun *et al.*[11] Immediately before surgery, intravenous blood alone (without anticoagulant) was collected (by venipuncturing of the antecubital vein) in two sterile 10-ml tubes and centrifugation was carried out immediately at 3000 rpm (approximately 400 g) for 10 min (R-4C, REMI, Mumbai, India). PRF the center layer was obtained after

centrifugation and separated from the other two layers above (platelets poor plasma) and below (red blood cell layer preserving a small portion of the same).

Intra-examiner calibration

To ensure adequate intra-examiner reproducibility, the examiner was calibrated before the beginning of the study. The examiners were considered calibrated once a statistically significant correlation and statistically non-significant difference between duplicate measurements were obtained ($r = 0.87$ for PD; $r = 0.91$ for RAL; $r = 90$ GML). PD, RAL and GML values were estimated to their nearest millimeter.

Surgical procedure

About 0.12% chlorhexidine digluconate was used as pre-surgical rinse. Iodine solution swab was used to carry out an extraoral antisepsis. After the administration of lignocaine 1:2,00,000 adrenalin local anesthesia, buccal and lingual sulcular incisions were made, and mucoperiosteal flaps were reflected. Maximum interproximal soft tissue was preserved. Root planning followed by debridement of the defect were carried out using ultrasonic instruments (EMS V-Dent, Shantou, Guangdong, China) and area-specific curets (Gracey curets, Hu-Friedy). No osseous recontouring was done. PRF of the required size was squeezed into the defects. Also, PRF of required size was used to cover the defect as a membrane. Repositioning of the mucoperiosteal flap was done and the flap was secured using a 3-0 non-absorbable silk suture (Ethicon, Johnson and Johnson, Somerville, NJ, USA). Interrupted sutures were placed. A periodontal dressing was placed in protection over the surgical site (Coe-Pak, GC America, Chicago, IL, USA). Post-operative instructions and suitable antibiotics and analgesics (Novamox LB 500 mg, twice per day; and Diclofenac three times a day, for 3 days) were prescribed.

Post-operative care

Patients were advised to rinse with chlorhexidine gluconate mouthrinse (0.12%) twice daily for a period of 15 days. At 1 week postoperatively, periodontal dressing and sutures were removed. Povidine-iodine solution was used to rinse the surgical site and the patients were instructed for gentle brushing with a soft toothbrush. Each patient was re-examined weekly up to 1 month after surgery and then at 3 and 9 months, and oral hygiene instructions were reinforced at each recall visit. No subgingival instrumentation was attempted at any of these appointments.

Post-surgical measurements

Soft and hard tissue evaluation was performed 9 months after surgery. Soft tissue measurements were repeated with previously used acrylic stents. For hard tissue reevaluation, second IOPA of the same study site was carried out and IBD measurement was reassessed at 9 months.

Primary and secondary outcome measures

The primary outcome of the study was bone defect fill evaluated radiographically. The secondary outcomes include changes in PD, CAL, mSBI and PI.

Statistical analysis

The data were analyzed using statistical software (SPSS v.20, IBM, Chicago, IL, USA). Power calculations were performed before the study was initiated. To achieve 90% power and detect mean differences of the clinical parameters between groups. The results were averaged (mean standard deviation) for each clinical and radiographical parameter at baseline and 9 months. mSBI and PI were expressed as absolute and relative counts and comparison was performed using Chi-square test.

Results

Wound healing was uneventful for all treated cases. Soft tissues healed within normal limits, and no significant visual differences were noted between the treatment groups. A statistically significant reduction in the PI and mSBI was observed in both the test and control sites at 9 months postoperatively. However, the difference between the test and control sites was statistically insignificant (Tables 1 and 2). Intra group and Inter group comparisons showed statistical significant reduction with PD and RAL and no difference was observed with GML levels (Tables 3 and 4). Statistically significant improvements were seen with the mean defect fill (cement enamel junction to base of the defect [CEJ-BOD] and AC-BOD) ($P = 0.003^*$) when intra group and inter group comparisons were made. However, no difference was observed in the distance from CEJ-AC (Tables 5 and 6).

Table 1: Intra group comparison of PI and SBI among study groups.

Parameters (N=20)	Groups	Visit	Mean±SD	P value
PI	Control	Baseline	1.324±0.387	0.005**
		9 months	0.492±0.174	
	Test	Baseline	1.442±0.496	0.005**
		9 months	0.583±0.209	
SBI	Control	Baseline	1.402±0.410	0.004**
		9 months	0.466±0.176	
	Test	Baseline	1.467±0.392	0.004**
		9 months	0.499±0.238	

PI: Plaque index, SBI: Sulcus bleeding index, SD: Standard deviation

Table 2: Intergroup comparison of PI and SBI among study groups.

Parameters (N=20)	Visit	Groups	Mean±SD	P value
PI	Baseline	Control	1.324±0.387	0.739
		Test	1.442±0.496	
	9 months	Control	0.492±0.174	0.393
		Test	0.583±0.209	
SBI	Baseline	Control	1.402±0.410	0.853
		Test	1.467±0.392	
	9 months	Control	0.466±0.176	0.912
		Test	0.499±0.238	

PI: Plaque index, SBI: Sulcus bleeding index, SD: Standard deviation

Table 3: Intragroup comparison of PD, RAL and GML among study groups.

Parameter (N=20)	Groups	Visit	Mean±SD	t value	P value
PD	Control	Baseline	6.20±0.632	6.000	0.000**
		9 months	4.60±0.699		
	Test	Baseline	5.90±0.994	8.143	0.000**
		9 months	4.00±0.816		
RAL	Control	Baseline	9.20±1.932	6.091	0.000**
		9 months	7.90±1.729		
	Test	Baseline	9.50±1.354	9.000	0.000**
		9 months	7.70±1.418		
GML	Control	Baseline	3.40±0.966	−1.406	0.193
		9 months	3.70±1.494		
	Test	Baseline	3.60±0.966	−1.964	0.08
		9 months	3.90±1.101		

*P<0.05 significant, **P<0.00 highly significant, PD: Pocket depth, RAL: Relative attachment level, GML: Gingival marginal level, SD: Standard deviation

Table 4: Intergroup comparison of soft tissue parameters between the study groups (in mm).

Clinical parameter (N=20)	Groups	Mean±SD	t value	P value
PD	Control	1.60±0.843	−0.847	0.408
	Test	1.90±0.738		
RAL	Control	1.30±0.675	−1.709	0.105
	Test	1.80±0.632		
GML	Control	−0.30±0.675	0.000	1.000
	Test	−0.30±0.483		

*P<0.05 significant, **P<0.00 highly significant, RAL: Relative attachment level, GML: Gingival marginal level, SD: Standard deviation

Table 5: Intra group comparison of mean defect fill (CEJ-BOD, AC-BOD, CEJ-AC among study groups.

Radiographic parameters (n=20)	Groups	Visit	Mean±SD	t value	P value
CEJ-BOD	Control	Baseline	7.80±2.201	9.750	0.000**
		9 months	6.50±1.856		
	Test	Baseline	8.30±2.058	7.471	0.000**
		9 months	5.70±1.229		
AC-BOD	Control	Baseline	3.70±1.059	7.236	0.000**
		9 months	2.90±0.966		
	Test	Baseline	3.95±0.497	9.222	0.000**
		9 months	2.50±0.527		
CEJ-AC	Control	Baseline	4.10±1.449	3.354	0.008**
		9 months	3.60±1.150		
	Test	Baseline	4.40±1.838	3.774	0.004**
		9 months	3.20±1.252		

*P<0.05 significant, **P<0.00 highly significant, CEJ-BOD: Cement enamel junction to base of the defect, AC-BOD: Alveolar crest to base of the defect, SD: Standard deviation

Table 6: Intergroup comparison of radiological parameters between the study groups (in mm).

Radiographic parameters	Groups	Mean±SD	t value	P value
CEJ-BOD	Control	1.30±0.422	3.488	0.003**
	Test	2.60±1.101		
AC-BOD	Control	0.80±0.350	3.382	0.003**
	Test	1.45±0.497		
CEJ-AC	Control	0.50±0.471	1.993	0.062
	Test	1.20±1.006		

*P<0.05 significant, **P<0.00 highly significant, CEJ-BOD: Cement enamel junction to base of the defect, AC-BOD: Alveolar crest to base of the defect, SD: Standard deviation

assessing the success of these treatment methods, complete closure of the defect is desirable. Therapeutic results can be measured by PD and RAL, bone regeneration, and evidence of histologic periodontal regeneration. Although histological evaluation is the most accurate method of evaluation, surgical closure of the defect and improvements in PD and RAL serve as suitable and practical outcome measures.[26]

The ideal goal for periodontal therapy is the reconstitution of bone and connective tissue attachment that has been destroyed by the disease process.[3] The uneventful healing in the patients is in agreement with our previous studies,[4,11,23-25] thus supporting the excellent properties of autologous PRF to enhance periodontal wound healing. Plaque, infection and smoking are the important factors that have been shown to significantly influence the outcomes of regenerative periodontal surgery.[27,28] Because the present study excludes smokers and only includes patients who were able to maintain acceptable oral hygiene, it may be assumed that the careful patient selection was also responsible for the positive outcomes obtained in both groups.

Only 3 and 2-wall IBDs were included because the number of remaining bony walls was found to be correlated positively with regeneration potential in grafting procedures.[29,30] Space maintenance is provided by the defect walls to minimize a membrane collapse and/or to provide the protection and retention of grafts.[31]

To the best of our knowledge, very few studies have reported the use of autologous PRF alone in the treatment of IBDs. This study is in accordance with our previous studies. However, the difference being our previous studies we included only 3-wall defects.

Conclusions
The addition of autologous PRF to OFD stimulated a significant improvement in the clinical and radiographic parameters and increase in bone fill compared to OFD alone at 9 months. However, long-term, multicenter histological studies are warranted to determine the precise effects of PRF on bone regeneration.

Discussion
The present study evaluated the clinical effectiveness of autologous PRF in treating vertical two and three walled intrabony defects. To avoid the effect of a natural variation between different individuals, each patient was treated using the split-mouth design (20 patients: 20 test sites: 20 control sites).

The treatment protocol emphasized the principles of careful soft tissue handling, wound stability, and infection control. In

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