Platelet-Rich Fibrin for the Treatment of Intrabony Periodontal Defects in Patients with Generalized Aggressive Periodontitis: A Randomized Controlled Clinical Study

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Abstract

Objective: Platelet-rich fibrin (PRF) is a fibrin matrix in which platelet cytokines, growth factors and cells are embedded; therefore, it has the potential to be used as regenerative therapy. The aim of this randomized clinical study was to evaluate the regenerative capacity of PRF when compared to collagen membrane (CM) for the treatment of intrabony periodontal defects in patients with generalized aggressive periodontitis (GAgP).

Methods: Using a split-mouth design, sixteen GAgP patients with paired contralateral intrabony defects were randomly assigned to the test group (composite bone graft {autogenous bone mixed with xenograft} + PRF, n = 16 sites) and control group (composite bone graft + CM, n = 16 sites). Plaque index, papillary bleeding index, probing pocket depth (PPD), clinical attachment level (CAL) and position of gingival margin (GML) were recorded prior to surgery and 6 months after surgery. The percentage of defect resolution (DR) was calculated radiographically at 6 months after surgery by intraoral periapical radiographs. Primary study outcome was change in CAL.

Results: Six-month results indicated that both treatment modalities resulted in a significant reduction in PPD, gain in CAL, and DR ($p < 0.05$). A statistically significant improvement in GML was recorded for the test group ($p < 0.05$) but not for the control. Intergroup comparison was insignificant for all parameters ($p > 0.05$) except for GML ($p < 0.05$).

Conclusions: Platelet-rich fibrin has shown favorable results that are comparable to CM for treatment of intrabony periodontal defects in patients with GAgP. However, better results concerning GML have been reported when PRF was used.

Keywords: Aggressive periodontitis, intrabony defects, platelet-rich fibrin, regenerative periodontal therapy

Introduction

One of the main objectives of periodontal therapy is regeneration of the lost supporting periodontal tissues (Polimeni *et al.,* 2006). The most positive outcome of periodontal regeneration procedures in intrabony defects has been achieved with a combination of bone graft and guided tissue regeneration (GTR) (McClain and Schallhorn, 1993; Guillemin, 1993). Bone grafts or bone graft substitutes have been combined with GTR, particularly with bioabsorbable membranes, with the rationale of supporting barrier membranes preventing collapse and promoting bone formation (Kim *et al.,* 2005).

Among the different available graft materials, autogenous bone remains the gold standard for osseous regeneration (Kim *et al.,* 2005; Deliberador *et al.,* 2006). Autogenous bone can be harvested from either extraoral or intraoral donor sites. Intraoral bone has a membranous origin and consequently has a lower resorption rate and enhanced vascularization. Moreover, intraoral bone harvesting is done in the dental office under local anesthesia and with relatively little morbidity. Despite its advantages (Precheur, 2007), only a limited amount of autogenous bone can be procured from intraoral sites,

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which may not be sufficient for complete fill of defects. Meanwhile, xenogenic grafts may represent a possible alternative to be mixed with autogenous bone for the treatment of intrabony defects (Hatano *et al.,* 2004).

A second-generation platelet concentrate, platelet-rich fibrin (PRF) was introduced by Choukroun *et al.* (2001). Platelet-rich fibrin is a matrix in which platelet cytokines, growth factors, and cells are embedded. It offers several advantages, including promoting wound healing, bone growth and maturation, graft stabilization and wound hemostasis (Choukroun *et al*., 2001). Moreover, it has minimum disadvantages in terms of antigenicity and cost. In periodontal infrabony defects, recent studies using PRF have shown good results as compared with open flap debridement (OFD) alone (Mazor *et al.,* 2009; Pradeep *et al.,* 2009). However, Lekovic *et al.* (2012) has compared PRF alone to PRF-bovine porous bone mineral (BPBM) combination. Six-month postsurgical measurements revealed a significantly greater reduction in probing pocket depth (PPD) and more attachment gain and defect fill in the PRF-BPBM group when compared with the PRF group. They concluded that although PRF can improve clinical parameters associated with human intrabony periodontal defects, a graft such as BPBM has the ability to augment the effects of PRF.

Generalized aggressive periodontitis (GAgP) is characterized by "generalized interproximal attachment loss affecting at least 3 permanent teeth other than first molars and incisors" (Lang *et al.,* 1999). The successful treatment of advanced periodontal destruction in patients with GAgP represents a significant challenge. Further investigations with combinations of current therapeutic choices with the best potential for hard and soft tissue regeneration are required.

Numerous studies have attempted to evaluate the effect of PRF in the treatment of intrabony defects (Lekovic *et al*., 2012). However, to the best of our knowledge, there are no published controlled clinical studies that compared the use of PRF/composite graft to collagen membrane (CM)/composite graft in periodontal intrabony defects. Hence, the aim of this study was to test the hypothesis that the efficiency of PRF for periodontal regeneration in intrabony defects is comparable with that of CM.

Patients and methods

Study design and patient selection

The study was carried out between December 2013 and April 2015. Two different approaches to treat intrabony periodontal defects were compared by using a split-mouth, randomized, single-blind controlled design. The same presurgical and surgical procedures were performed in all patients. The application of either PRF or CM (Biocollagen, Healiguide, Advanced Biotech Products (P) Ltd., Encoll Corp., Fremont, CA, USA) to cover a composite bone graft (autogenous bone mixed with xenograft) was the only difference between the groups. One investigator who was blinded to the surgical procedures assessed the clinical measurements. The study was designed in accordance with the Declaration of Helsinki of 1964, revised in Tokyo 2004 and was approved by the institutional review board.

The study population was recruited among patients referred to the Department of Oral Medicine and Periodontology, October 6 University (Egypt) and had a provisional diagnosis of GAgP (Armitage, 1999). Patients who had any medical condition or were on therapeutic regimen that could decrease the probability of soft tissue or bone healing, smokers, pregnant or lactating women, one-walled defects, patients with parafunctional habits and who had periodontal surgery in last 6 months were excluded. Non-vital teeth were also excluded from the study.

Following confirmation of the diagnosis, all patients underwent comprehensive periodontal examination and phase I therapy. A periodontal re-evaluation after 4 - 6 weeks was performed to determine the patient's response to the initial therapy and confirm eligibility for the study. Sixteen patients having radiographic evidence of paired contralateral vertical defects ≥ 4 mm (two- or three-walled) along with an inter-proximal PPD ≥ 6 mm at the experimental sites and showing good oral hygiene (plaque index (PI) < 1; Silness and Löe, 1964) were selected for the study. Informed consent forms were obtained after a detailed explanation of the purpose of the study and before baseline measurements were taken. Clinical and radiographic outcomes were measured on the day of surgery and 6 months postoperatively.

Preparation of platelet-rich fibrin

A volume of 10 ml of blood was drawn from each participant through venipuncture of the right arm and placed in sterilized vacuum-evacuated vials without an anticoagulant and centrifuged immediately at 400 x *g* for 10 min, according to the protocol developed by Choukroun *et al.* (2006), using a tabletop centrifuge (Shanghai Medical Instruments, China).

Surgical procedures

At the time of the surgical procedure, subjects were randomly allocated using a previously made computer-generated list to one of the following treatment groups: Composite graft + PRF (test group); composite graft + CM (control group).

A researcher not involved in the examinations and surgical procedures conducted the allocation concealment. After local anesthesia, buccal and lingual intrasulcular incisions were made with vertical releasing incisions when needed and full thickness mucoperiosteal flaps were reflected.

Alveolar bone was exposed > 3 mm beyond the defect margin. Meticulous debridement using Gracey's area specific curettes (Hu-Friedy, Chicago, IL, USA), root planing and copious irrigation by sterile saline was performed. Afterwards, the defect's configuration was evaluated to ensure meeting the inclusion criteria. Bone chips were harvested by repeatedly drawing a bone scraper (Mr. Curette Co.) over the exposed bone surface near the operating area. A composite graft was obtained by mixing autogenous bone chips with xenograft (Biogen, Healiguide, Advanced Biotech Products (P) Ltd., Encoll Corp., Fremont, CA, USA) in a sterile dappen dish. Incremental endosseous defect filling with the graft was performed. In the control group, CM was trimmed according to the defect's configuration, and then adapted over the defect filled with graft material coronal to the interproximal bone crest and extending 3 mm apically and laterally over the adjacent bone. Care was taken to avoid the overfilling of the defect so as to ensure adequate closure of the flap. A periosteal releasing incision was performed to assure complete membrane coverage. At the test site, the defects received PRF instead of CM. The flaps were coronally advanced to obtain complete coverage of the defect. Care was taken to secure an adequate tension-free interproximal closure. Suturing was done with interrupted 4-0 silk sutures and a periodontal dressing was placed. A highly experienced single surgeon performed all treatments.

All patients were given written postoperative instructions and prescribed the analgesic diclofenac sodium 50 mg twice a day for 3 days, amoxycillin 500 mg three times daily for 5 days, and chlorhexidine mouth rinse (0.12%) twice daily for 6 weeks. Mechanical tooth cleaning was not allowed in the surgical area for the first 4 weeks postoperatively. The sutures were removed 14 days after surgery. Recall appointments for professional supragingival tooth cleaning and oral hygiene reinforcement were scheduled every other week during the first 2 months after surgery, and once a month for the rest of the study period.

Clinical parameters

The following clinical parameters were recorded just before surgery as baseline data, and then at 6 months post-surgery: PI (Silness and Löe, 1964), papillary bleeding index (PBI; Muhlemann, 1977), PPD, CAL and position of gingival margin (GML). All measurements were recorded using William's graduated periodontal probe and rounded up to the nearest millimeter. Probing pocket depth and CAL were measured at six sites per tooth. The deepest point in each defect was considered for statistical analysis.

Radiographic evaluation

Periapical radiographs were taken at baseline and 6 months postoperatively for each defect. The radiographs were digitized by means of a scanner and fed to a computer-imaging device that recognized the scanner signal. By means of an image analysis program (PorDios, Institute of Orthodontic Computer Science Ltd., Aarhus, Denmark; Gotfredsen, 1999), the following parameters were estimated on the images: a) the distance from the cementoenamel junction (CEJ) to the bottom of the intrabony defect (BD), representing the radiographic bone level (RBL); b) the distance from the CEJ to the bone crest (CBL); and c) the distance from the bone crest to BD, representing the intrabony component (IC) of the defect (*Figure 1*). The differences were calculated as height of bone fill and crestal bone resorption. The percentage defect resolution (DR) was calculated by the formula: ((IC baseline - IC 6 months) × 100)/IC baseline**.**

Statistical analysis

Each defect was considered as a statistical unit. Primary study outcomes were changes in CAL at the six-month evaluation period. Descriptive data that included mean ± standard deviation (SD) were calculated for clinical and radiographic parameters at baseline and 6 months. Following this, the data were subjected to statistical analysis using the Wilcoxon signed rank test for intragroup comparison and the Mann-Whitney U test for intergroup comparison. The level of significance was set at the probability value $p \leq 0.05$. All calculations were performed using SPSS data analysis software (Ver.14.0; SPSS Inc.).

Results

Sixteen patients (6 males and 10 females, mean age 24 ± 3.3 years, range 20 - 35 years) completed the study. All tolerated the surgical procedures and no postoperative complications or adverse events were seen with any of the participants during the study period. Nevertheless, three cases treated by CM exhibited mild membrane exposure 2 weeks after surgery with minimal inflammatory response.

A statistically significant improvement was observed in both groups in terms of PPD, CAL and percentage DR ($p < 0.05$, *Tables 1, 2*). No intergroup statistically significant differences in the mean values of PPD, CAL, DR% were found at the 6-month evaluation period ($p > 0.05$, *Tables 1, 2*).

The mean PPD values were 7.88 ± 0.8 mm and 7.88 ± 0.93 mm for the test and control sites respectively. At 6 months, the mean PPD values significantly decreased to 3.96 ± 0.69 mm and 3.96 ± 0.62 mm respectively. At baseline, the mean CAL value was 8.79 ± 1.03 mm and 8.71 ± 1.01 mm for the test and control sites respectively. At 6 months, the mean CAL value decreased to 4.29 ± 0.75 mm and 4.67 ± 0.69 mm respectively (*Table 1*). A defect resolution of 51 ± 13.01% was obtained in the control group while the corresponding value was 53 ± 10.5% in the test group. The difference between groups was statistically insignificant ($p > 0.05$; *Table 2*, *Figures 1*, *2*).

Table 1. Clinical parameters at baseline and at 6-month evaluation

Parameter	Test group (composite bone graft + PRF, n = 16 sites)	Control group (composite bone graft + CM, n = 16 sites)	*p* value**
PI			
Baseline	0.76 ± 0.13	0.70 ± 0.15	
6 months	0.76 ± 0.12	0.72 ± 0.16	$p > 0.05$
p value*	0.90	0.90	
PBI			
Baseline	0.63 ± 0.13	0.65 ± 0.15	
6 months	0.48 ± 0.1	0.53 ± 0.13	$p > 0.05$
p value*	0.003***	0.005***	
PPD (mm)			
Baseline	7.88 ± 0.8	7.88 ± 0.93	
6 months	3.96 ± 0.69	3.96 ± 0.62	$p > 0.05$
p value*	0.002***	0.002***	
CAL (mm)			
Baseline	8.79 ± 1.03	8.71 ± 1.01	
6 months	4.29 ± 0.75	4.67 ± 0.69	$p > 0.05$
p value*	0.002***	0.002***	
GML (mm)			
Baseline	1.08 ± 1.14	1.08 ± 1.08	
6 months	0.62 ± 0.77	0.79 ± 0.84	$p < 0.05$
p value*	0.036***	0.33	

*Intragroup difference at 6 months analyzed by Wilcoxon signed rank test; **intergroup difference at 6 months analyzed by Mann Whitney U test; ***statistically significant. PI, plaque index; PBI, papillary bleeding index; PPD, probing pocket depth (mm); PRF, platelet-rich fibrin; CAL, clinical attachment level (mm); CM, collagen membrane; GML, gingival margin level

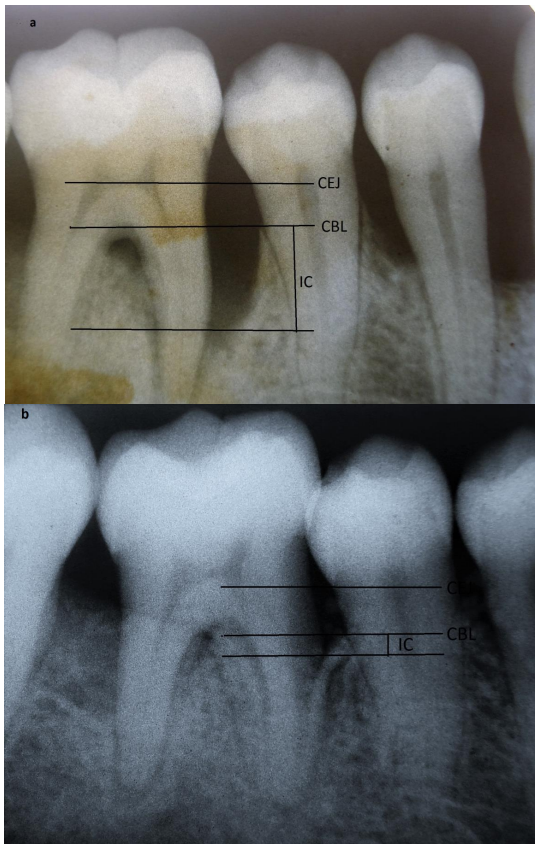

Figure 1. Test group: a) Pre-operative and b) post-operative radiographs. CEJ, cementoenamel junction; CBL, crestal bone level; IC, intrabony component

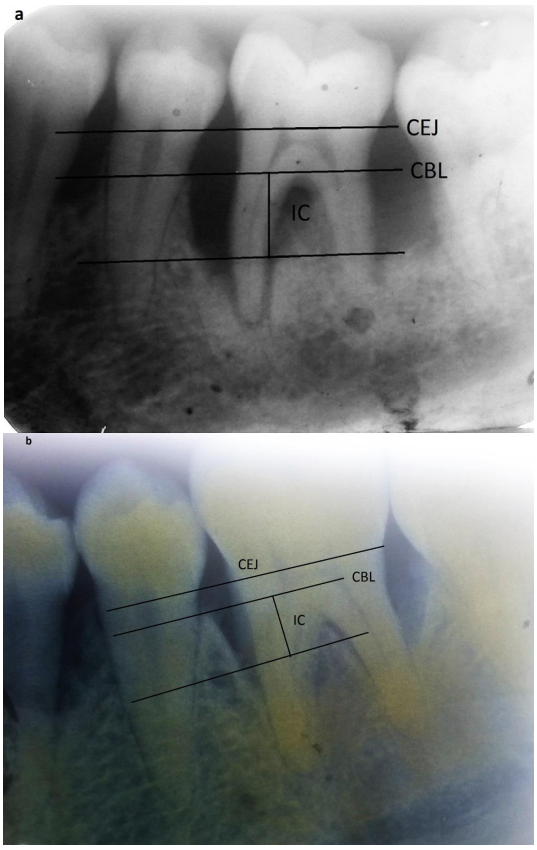

Figure 2. Control group: a) Pre-operative and b) post-operative radiographs. CEJ, cementoenamel junction; CBL, crestal bone level; IC, intrabony component

Table 2. Radiographic parameters at baseline and at 6-month evaluation

	Test group		Control group	
IC	**Baseline**	**6 months**	**Baseline**	**6 months**
(RBL-CBL)	6.79 ± 1.93	3.29 ± 1.00***	6.28 ± 1.89	3.15 ± 1.01***
DR%	53 ± 10.5%		51 ± 13.01 %	

***Statistically significant ($p \leq 0.05$) by Wilcoxon signed rank test. IC, intrabony component; RBL, radiographic bone level; CBL, crestal bone level; DR, defect resolution

No significant change in GML was recorded for the control group at the 6-month evaluation period ($p = 0.33$). However, for the test group, GML significantly improved from 1.08 ± 1.14 mm to 0.62 ± 0.77 mm ($p = 0.036$). The difference between groups was statistically significant ($p < 0.05$).

There was no statistically significant difference in PI from baseline to 6 months following surgery for any group. However, a statistically significant difference in the PBI scores from baseline to 6 months following surgery was reported. The difference between groups was statistically insignificant ($p > 0.05$; *Table 1*). All PI and PBI scores were within clinically healthy parameters.

Discussion

Platelet-rich fibrin consists of an intimate assembly of cytokines, glycan chains, and structural glycoproteins enmeshed within a slow releasing fibrin network. It shows complex architectures as a healing matrix, including mechanical properties that no other platelet concentrates can offer. This leads to more efficient and sustained cell migration and proliferation and introduces PRF as a carrier for cells that are essential for tissue regeneration (Lundquist *et al.,* 2008). He *et al.* (2009) reported the superiority of PRF in alkaline phosphatase expression and induction of mineralization when compared with platelet-rich plasma *in vitro*. Moreover, PRF has been shown to act as suitable scaffold for cultivating human periosteal cells *in vitro*, which may be suitable for applications in bone tissue engineering (Gassling *et al.,* 2010). The present study was conducted to evaluate the efficacy of PRF in GTR procedures.

Comparing a graft material to open flap debridement for any study purpose does not seem to be ethical because we intentionally leave a site without grafting where it is indicated. Moreover, despite many studies reported that PRF can be used alone (Mazor *et al.,* 2009, Pradeep *et al.,* 2009, Ozdemir *et al.,* 2013) or in combination with different bone substitutes (Lekovic *et al.*, 2012), the best regenerative results were obtained with a combination of bone graft and GTR. Therefore, we have clinically and radiographically compared the effectiveness of composite bone with PRF (test group) with composite bone and CM (control group) in the treatment of human intrabony periodontal defects in GAgP patients with the intention of performing the technique with the best regenerative potentials in such difficult cases. To limit the patient-based confounding factors, the split mouth design was used with comparable defect characteristics.

The use of autogenous bone grafts is considered to be the best choice for reconstructive surgery. Several methods are available for harvesting particulate bone (Hatano *et al.,* 2004, Artzi *et al.,* 2005, Le Lorc'h-Bukiet *et al.,* 2005).The most common method is to mill large bone portions (Le Lorc'h-Bukiet *et al.,* 2005). However, bone milling by rotating electrical instruments may reduce the amount of viable bone cells (Springer *et al.,* 2004). Moreover, bone harvesting may be exposed to microbial contamination (Young *et al.,* 2001, Young *et al.,* 2002). Harvesting intraoral autogenous bone by the use of bone scraper, as in the current study, is less aggressive. Further, bone scraping harvested good quantities of uncontaminated autologous bone suitable for grafting closed the donor site. This shortens the operating time and minimizes the invasiveness of the procedure.

No uneventful healing or postoperative complication was observed in either group. This is in agreement with previous studies of Sharma and Pradeep (2011) and Thorat *et al.* (2011), confirming the biocompatible nature of PRF. Platelet-rich fibrin might decrease harmful effects of the inflammatory processes mainly by correcting certain destructive and noxious excesses during the healing process (Dohan *et al.,* 2006).

Only patients maintaining acceptable oral hygiene (PI < 1) were included. Statistically non-significant ($p > 0.05$) change in mean PI for both groups at the 6-month evaluation period reflects compliance with oral hygiene instructions. This is an important issue in regenerative periodontal surgery and may be partially responsible for the favorable outcome obtained in the current study. A statistically significant difference in the PBI scores from baseline to 6 months following surgery was reported. However, both values were within normal range.

In agreement with previous studies (Sharma and Pradeep, 2011; Thorat *et al.*, 2011), statistically significant reductions in PPD and gains in CAL were reported for both groups. The mean reduction in PPD was about 4 mm in test and control sites. There was a mean CAL gain of 4.5 mm and 4 mm in test and control groups, respectively. This finding is in agreement with the recent systematic review (Parrish *et al.,* 2009), which showed that intrabony defects treated with CM with graft material resulted in a mean CAL gain of 3.48 mm, with a range of 2.3 mm to 4.1 mm. The results obtained in the test group may be attributed to sustained release of growth factors by PRF and its complex architecture as a healing matrix (Choukroun *et al.*, 2001).

Statistically significant defect resolution of about 51% and 53% was observed in control and test groups, respectively, after 6 months of intervention. The slightly higher DR in the test group may be attributed to osteoinductive effect of growth factors enmeshed within PRF. The DR is in agreement with the gain in the CAL. It is difficult to compare measurements of DR in the present study with previous studies because of mode of measurement. In the majority of earlier studies, re-entry measurements were made, whereas radiographic interpretation was used in this study. Re-entry surgery was not performed for ethical concerns and the probability of further crestal alveolar bone loss (Singh *et al.,* 2000).

An insignificant change in the level of GML was observed after 6 months in the CM-treated sites. However, unlike previous studies (Sharma and Pradeep, 2011; Thorat *et al*., 2011), a significant change in GML (1.08 ± 1.14 to 0.62 ± 0.77 mm) was reported for the PRF group. Inter-group comparison revealed more gingival recession in the control group, and the difference was statistically significant. This result is suggestive of the potential value of PRF in the management of gingival recession.

Within the limitations of this study it can be concluded that use of PRF in conjunction with composite bone graft resulted in significant improvement in clinical and radiographic parameters. The results were comparable to what can be achieved by CM with composite bone graft. However, the PRF group achieved better results regarding changes in GML. Further studies with a larger sample size and long-term observations would verify the findings presented here. Moreover, as histology is the ultimate standard to assess periodontal regeneration, histological studies are necessary to assess the regenerative potential of PRF.

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