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THE EFFICACY OF COMBINING ADVANCED PLATELET-RICH FIBRIN TO BIPHASIC ALLOPLAST IN MANAGEMENT OF INTRABONY DEFECTS (RANDOMIZED, CONTROLLED, CLINICAL TRIAL)

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ABSTRACT

INTRODUCTION: Advanced platelet-rich fibrin (A-PRF) is one of the most recently developed platelet concentrates and believed to have a great impact in regeneration for its richness in leukocytes, platelets, stem cells and cytokines. Biphasic calcium phosphate (BCP) alloplast is a well-known synthetic bone substitute widely used in periodontal regeneration.
OBJECTIVES: To evaluate the additive effect of A-PRF to BCP in regeneration of intrabony defects.
MATERIALS AND METHODS: Twenty two interproximal, intrabony defects in 22 patients with moderate to severe chronic periodontitis were allocated to one of the two groups of this study. Group I (test) was treated with open flap debridement followed by placing A-PRF and BCP with a ratio of 1:1, while group II (control) was filled only by BCP mixed with saline after open flap debridement (OFD). Plaque index (PI), modified gingival index (MGI), probing depth (PD) and clinical attachment level (CAL) were evaluated at baseline, 3, 6 and 9 months.
RESULTS: Compared to baseline all evaluated parameters showed improvement at the end of the study in both groups. Changes from baseline to 9 months in A-PRF/BCP group were insignificantly greater compared to BCP/saline group regarding reduction of PI (0.54±0.72 vs 0.53±0.92), MGI (0.34±0.68 vs 0.32±0.78), PD (2.27±0.71mm vs 2.04±0.96mm) and CAL gain (2.13±1.02mm vs 1.68±1.23mm). However, intergroup differences were insignificant.
CONCLUSIONS: Based on results of this study, combining advanced platelet-rich fibrin to biphasic alloplast mixture was more advantageous than using BCP alone in intrabony defect management.
KEYWORDS: Advanced platelet-rich fibrin, biphasic alloplast, intrabony defects, periodontal management.

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INTRODUCTION

Periodontal diseases are one of the major oral problems that require special attention and professional management (1). Chronic periodontitis is one of the most prevalent periodontal diseases (2) which is characterized by clinical attachment loss, alveolar bone destruction and eventually bone deformities and defects (3, 4). These intrabony defects (IBD) usually aggravate the condition and worsen the prognosis, as they act as plaque retentive areas and complicate treatment (5).

Several therapeutic modalities have been introduced to eliminate IBDs aiming for regeneration of lost periodontal tissues. Among these treatments were guided tissue regeneration (GTR) (6), enamel matrix derivatives (EMD)(7), growth factors (8), with or without bone grafts (9).

Interest in platelets concentrates (PC) rapidly increased over the past few years in all fields of medicine due to their healing potentials as they are rich in platelets and cytokines crucial for regeneration (10). However, the first generation of platelets concentrate, platelet-rich plasma (PRP) had several shortcomings, which included the need to use bovine or artificial anticoagulant in its preparation leading to hypersensitivity reactions and healing impairment. Additionally, the technique sensitive preparation protocol required several centrifugation steps and speeds (11, 12).

In 2001 Choukroun et al,(13) introduced the first platelets-rich fibrin (L-PRF) that made a revolution in the concept of (PC) as it overcame all drawbacks of PRP. Ever since modifications have been proposed to the preparation protocol to maximize the regenerative capacity. The one alteration that had greatest impact was the reduction of centrifugation speed, as it resulted in a significant rise in leukocytes, platelet counts and eventually released growth factors (14). The solid form that resulted from this low-speed concept , called advanced platelets-rich fibrin (A-PRF), was presented in 2014 by Choukroun et al, (15).

This A-PRF is considered one of the most regenerative versions of PRF as it has the highest concentration of WBCs and platelets, also studies have proved that it shows the greatest growth factors release when compared to PRP, concentrated growth factors (CGF) and leukocyte platelet-rich fibrin (L-PRF) (16, 17). These growth factors include transforming growth factor-beta1 (TGF-β1), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), which regulate bone formation and the healing process in general. Another advantage of A-PRF was the slow sustained release of cytokines unlike the rapid liberation of PRP (18). This property allows A-PRF to stimulate the local environment for longer periods of time, thus ensuring more potent effect regarding regeneration (19).

Numerous bone grafts and their substitutes have been successfully used in grafting procedures in intrabony defects. They include autografts, allografts, xenografts and alloplasts. Synthetic alloplasts provide a remarkable advantage over other types of bone grafts, as they lack the risk of disease transmission, donor site morbidity, and provide unlimited source of grafting material (20, 21).

Data on the clinical effect of A-PRF and BCP composite is insufficient. The aim of the current study was to evaluate the clinical effect of combining A-PRF with BCP and assess the impact of this composite in the regeneration of intrabony defects.

MATERIALS AND METHODS

A. Materials:

Sample size:

Twenty two defects in twenty two patients (12 females and 10 males) with moderate to severe chronic periodontitis were selected for this study. This sample size was the enough required sample as statistically significant with 80% power and at a significance level of 95% (accepted α error = 0.05). All patients were collected from the outpatient clinic in Oral Medicine, Periodontology, Oral Diagnosis and Radiology Department, Faculty of Dentistry, Alexandria University, Egypt. All subjects approved to participate in the clinical trial and signed a written informed consent. The study was accepted by the Research Ethics Committee of the faculty of Dentistry, Alexandria University (IRB NO: 00010556-IORG0008839). It also followed the principles of the modified Helsinki code for human clinical studies (2013) (22) and CONSORT 2010 guidelines for reporting randomized clinical trials (23).

Study design:

The study consisted from two equal groups (eleven defects per group).

Patients in group I (Test) were treated using A-PRF/BCP after proper open flap debridement (OFD), while group II (control) were treated with BCP/saline after OFD. Patient selection was conducted according to the following inclusion and exclusion criteria.

Inclusion criteria included systemically healthy patients of both sexes, with an age ranging from 25 to 55 years old, with no history of previous periodontal surgery at defect site during the past six months. Presence of interproximal defects with probing depth (PD) ≥5mm, clinical attachment loss (CAL) ≥4 mm and radiographic evidence of the presence of intrabony defect ≥3 mm in depth as revealed by panoramic radiographs. While the exclusion criteria included uncooperative patients who failed to maintain an O'Leary plaque index (24) ≤10% after phase I therapy, aggressive periodontitis patients and those with systemic conditions that may affect periodontal treatment such as uncontrolled diabetes mellitus or deleterious habits such as smoking, as well as pregnant and lactating females. Non-vital teeth or with grade III mobility were also excluded.

Grafting materials:

1-Bipahsic alloplast (METABIOMEND©): META BIOMEND Co. LTD © Korea

A synthetic biphasic calcium phosphate (BCP), composed of (40%) Beta tricalcium phosphate (β-TCP) and (60%) hydroxyapatite crystals (HA) with particle size varying from 0.5 to 1 mm. (Fig.1)

2-Advanced platelet-rich fibrin (A-PRF):

A recently introduced autologous biomaterial composed of dense fibrin matrix filled with platelets, white blood cells, stem cells and growth factors. Preparation protocol will be discussed in method section. (Fig. 2)

Figure 1: METABIOMEND biphasic calcium phosphate
Figure 2: A-PRF clots formed within plain glass tubes

B. Methods:

1-Phase I therapy:

Oral hygiene instructions were provided to all patients, and full mouth scaling and root planning using hand and ultra- sonic scalers was performed. Four weeks later, patients were recalled for re-evaluation to confirm suitability for surgery. Only those patients accomplishing an O'Leary plaque index (24) ≤10% were recruited in the study.

2- Pre-surgical measurements:

Site specific clinical parameters measured at baseline and all following follow up visits were measured by the same examiner from buccal and lingual surfaces at defect site. They included probing depth (PD)(25) measured from gingival margin till the depth of the pocket and clinical

attachment level (CAL)(25) measured from cemento-enamel junction (CEJ) till the depth of the pocket. Both parameters were measured using graduated periodontal probe with William's calibration (Nordent, New York, USA) in the presence of custom-made acrylic stents with grooves to ensure reproducibility of probe placement at each time. Plaque index (PI)(26) and modified gingival index (MGI)(27) were also measured.

3- A-PRF preparation:

Advanced platelet-rich fibrin was prepared according to the protocol developed by Choukroun et al(15). Fresh blood was collected from the patients' brachial veins to fill 10 ml plain glass tube without anticoagulants. Blood samples were then centrifuged using angled benchtop centrifuge (Tangulaw 1-800 China) at 200g for 8 minutes. After centrifugation A-PRF membranes were collected with sterile straight forceps, compressed using PRF box (Cynamed, Germany) to squeeze out excess serum. Finally, A-PRF membranes were cut in small pieces using sterile scissors.

4- Surgical procedure:

Under local anesthesia, gingival sulcular incisions were made. Then buccal and lingual flaps were reflected using periosteal elevators (Kohler, Southern Germany). Meticulous defect debridement as well as root planing were done using hand scalers and curettes. In the test group minced A-PRF was mixed with BCP in a 1:1 ratio (by volume) then placed into the defect till the alveolar crest of bone. While, in control group BCP was mixed with sterile saline and placed into the defect also till the highest point of the alveolar crest without overfilling. Finally, flaps were approximated and secured with absorbable 4/0 vicryl sutures (Ethicon, Johnson& Johnson, USA). (Figures 3-5)

5- Post operative care:

Patients were given postoperative oral hygiene instructions and medications including Amoxicillin Clavulanate (Augmentin, Medical Union Pharmaceutical, Egypt)1 gm twice daily for 7 days, ibuprofen (Brufen, CPCI, Egypt) 600 mg, twice daily, anti-inflammatory (Alphentern, Amoun Pharmaceutical Co., Egypt) drug 1 tablet 3 times daily, and chlorohexidine digluconate (CHX) mouth rinse (Hexitol, ADCO, Egypt) (0.12%) twice daily. Sutures were removed 10 days after surgery.

6- Post operative measurements:

Site specific PI, MGI, PD and CAL were measured using the same acrylic stent used preoperatively.

7- Statistical analysis:

Data were reviewed to check for any errors during data entry. Descriptive statistics were performed using frequencies and percentages for qualitative data while mean and standard deviation (SD) were used for quantitative data. Graphical presentation was done using bar charts and line graphs.

Normality was checked using descriptive statistics, plots (histogram and box plot) and Shapiro-Wilk test. All inter-group comparisons were analyzed using a parametric test; Student's independent t test, while Mann-Whitney U test was used when the variables were not normally distributed. Intra-group comparisons were assessed by using Paired t test for the normally distributed variables and Wilcoxon Sign Rank test was applied for those not normally distributed(28, 29). Mean difference was computed according to the formula (Baseline-follow up point of time). Percentage change was calculated according to the formula [(after-before)/before] x 100. Significance was set at $P \leq 0.05$. Data was analyzed using IBM SPSS statistical software (version 23) (IBM SPSS Collaboration and Deployment Services)(30).

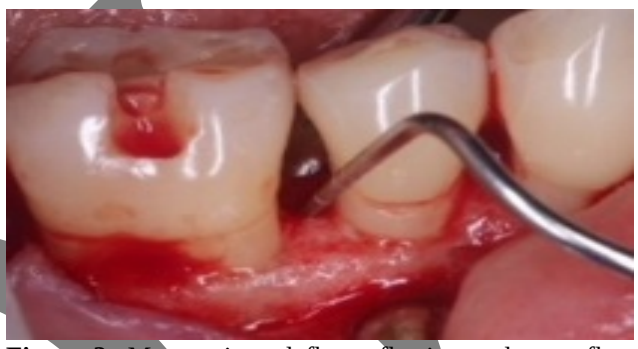

Figure 3: Mucoperiosteal flap reflection and open flap debridement of intrabony defect mesial to lower right 6 in subject no. 8 in test group.

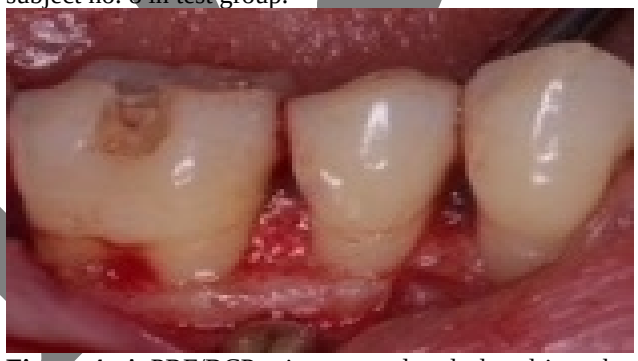

Figure 4: A-PRF/BCP mix prepared and placed into the defect.

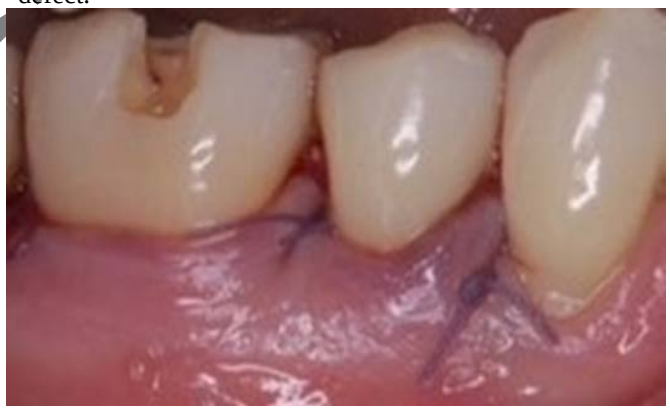

Figure 5: Flap repositioning and suturing with simple interrupted sutures.

RESULTS

All twenty two enrolled patients completed the study. Table1 demonstrates the mean of all clinical variables as well as their intergroup comparison. While Table 2 shows changes in clinical parameters in both groups from baseline to all follow up periods.

At baseline no significant difference was seen between two groups in all parameters. By the end of the study at 9 months, intragroup reduction of plaque index was statistically significant in both A-PRF/BCP (0.54±0.72) and BCP/saline (0.53±0.92) groups with (P value = 0.03 and 0.04) respectively. However, no significant difference in mean PI was seen between the two groups at all-time points. Although the decrease in mean modified gingival index (MGI) at 9 months was greater in test group than in control group (0.34±0.68 vs 0.32±0.78), the difference was not statistically significant in either groups or between them.

Concerning the PD, significant reduction from baseline was present in both groups at all-time intervals. However, no significant difference was seen between the two groups at any time point. By the end of study, mean PD decreased from (5.72±0.98mm) to (3.45±0.93mm) in A-PRF/BCP group, and from (6.22±1.34mm) to (4.18±0.81mm) in BCP/saline group. Similarly, CAL gain was statistically significant throughout the study in both groups but not between them. Mean CAL in test group decreased from baseline (5.22±1.25mm) to 9 months (3.09±1.24mm), while control group had less improvement from baseline (5.72±1.55mm) to 9 months (4.04±1.03mm). At 9 months, PD reduction and CAL gain were greater in A-PRF/BCP group on expense of BCP/saline group.

Table1: Mean PI, MGI, PD, CAL with intergroup comparison throughout the study

Evaluated parameters		baseline	3 months	6 months	9 months
Plaque index PI	A-PRF/BCP	1.00±0.83	0.81±0.75	0.63±0.49	0.45±0.56
	BCP/saline	1.18±0.84	0.95±0.65	0.86±0.77	0.64±0.56
	Statistical significance	0.61	0.61	0.56	0.65
Modified gingival index MGI	A-PRF/BCP	0.55±0.52	0.40±0.84	0.24±0.45	0.15±0.41
	BCP/saline	0.77±0.68	0.67±0.63	0.52±0.85	0.45±0.66
	Statistical significance	0.42	0.62	0.61	0.67
Probing depth PD in mm	A-PRF/BCP	5.72±0.98	4.22±1.05	3.77±0.95	3.45±0.93
	BCP/saline	6.22±1.34	4.27±1.61	4.04±1.40	4.18±0.81
	Statistical significance	0.33	0.93	0.60	0.06
Clinical attachment level CAL in mm	A-PRF/BCP	5.22±1.25	3.86±1.12	3.45±1.31	3.09±1.24
	BCP/saline	5.72±1.55	4.09±1.22	3.77±1.53	4.04±1.03
	Statistical significance	0.41	0.63	0.66	0.06

DISCUSSION

All recent therapeutic modalities aim to achieve periodontal regeneration through restoring lost bone, cementum and periodontal ligament tissues. Interest in autologous blood concentrates has been rapidly increasing over the past few years for their high regenerative potentials.

Evaluated parameters/ duration		Baseline-3months	Baseline-6months	Baseline-9months
Plaque index PI	A-PRF/BCP	0.18±0.92 NS	0.36±0.76 NS	0.54±0.72 S
	BCP/saline	0.22±0.60 NS	0.31±1.10 NS	0.53±0.92 S
Modified gingival index MGI	A-PRF/BCP	0.15±0.84 NS	0.31±0.73 NS	0.34±0.68 NS
	BCP/saline	0.10±0.93 NS	0.25±1.10 NS	0.32±0.78 NS
Probing depth (PD) reduction in mm	A-PRF/BCP	1.50±0.74 S	1.95±0.56 S	2.27±0.71 S
	BCP/saline	1.95±0.90 S	2.18±1.27 S	2.04±0.96 S
Clinical attachment level (CAL) gain in mm	A-PRF/BCP	1.36±1.16 S	1.77±0.68 S	2.13±1.02 S
	BCP/saline	1.63±0.89 S	1.68±1.23 S	1.68±1.23 S

Table 2: Change in clinical parameters from baseline to each time interval

Advanced platelet-rich fibrin (A-PRF) is considered one of the most recent platelets concentrates. It has several superior properties than its previous precursors, which can add to periodontal regeneration. In this study we evaluated the effect of A-PRF in periodontal regeneration of intrabony defects.

All patients experienced uneventful healing indicating that all the used biomaterials well tolerated. Improvement in mean plaque index (PI) from baseline to 9 months was better in test group (0.54±0.72) than the control group (0.53±0.92). Both groups had statistically significant reduction in PI, but without intergroup significance. At 9 months both groups showed improvement in the mean modified gingival index (MGI) with significant difference between A-PRF/BCP group and BCP/saline group (0.21±0.41 and 0.45±0.66).

These results are in accordance with those of Pradeep et al,(31) Ashawan et al,(32) and Agrawal el al,(33). However , all these authors used gingival index (GI)(26) rather than modified gingival index (MGI)(27), which was preferred in this study for its non-invasiveness.

The decrease in these indices indicates the healthy gingival condition in subjects during the study, which can be explained by proper oral hygiene measures and their reinforcement during the follow up visits.

Although change in PD from baseline to each follow up was statistically significant in each group separately, no significant difference was found between the two groups at any time interval. Reduction in PD from baseline to 9 months was greater in A-PRF/BCP group (2.27±0.71) than in BCP/saline group (2.04±0.96).

Similarly, statistically significant difference in CAL gain was seen from baseline to each follow up in both groups individually, but not between the two groups. The gain in (CAL) from baseline to 9 months was greater in A-PRF/BCP group (2.13±1.02) than in BCP/saline group (1.68±1.23).

To the best of our knowledge, clinical studies using A-PRF are scarce, and therefore our results were compared to other resembling studies evaluating other forms of platelet concentrates and bone grafts in periodontal regeneration.

PD and CAL gain in the current study were comparable to those of Naqvi et al,(34) 2017 who found that PD reduction and CAL gain were greater in test group (bioactive glass /PRF) (3.2±2.3 mm and 4.1±1.73 mm) than control group (bioactive glass alone) (3.15±1.06 mm and 3.15±1.06 mm) without significant difference between the two groups. Similarly, Bodhare et al,(35) found significant reduction in PD in both groups (PRF/bioactive alloplast and bioactive alloplast alone), but without intergroup significance. However, significant difference was seen between the two groups in mean CAL gain from baseline to 6 months. In 2015, Agarwal et al,(36) found significant difference between the two groups when they compared PRF/DFDBA to DFDBA alone in intrabony defects. In another trial with study design similar to ours, Sezign et al,(37) evaluated the effect of PRF combined with anorganic bovine bone material (ABBM) versus ABBM alone in intrabony defects, their results showed improvement in all parameters, but without significant difference between the two groups except for CAL gain.

The favorable results with insignificant difference between to two groups in PD reduction and CAL gain, indicate the ability of BCP to induce regeneration in intrabony defects. This is supported by previous studies conducted by Stein et al,(38) Lee et al,(39) and Stevanovic et al,(40) who compared the regenerative capacity of biphasic alloplast in intrabony defects to open flap debridement (OFD), other alloplast (β-TCP), autograft and xenograft (Bio-oss). Their results showed that PD reduction and CAL gain were significantly higher in biphasic alloplast groups than OFD and β-TCP, and similar to those of Bio-oss and autograft.

Superior results seen in A-PRF/BCP group can be explained by the presence of tremendous amounts of platelets, leukocytes and various growth factors (GF) associated with healing and regeneration such as transforming growth factor-beta1 (TGF-β1), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) embedded within dense fibrin network.

CONCLUSION

Within the limitation of this study we can conclude:

1- Both treatment modalities can be used in management of intrabony defects.
2- Combination of A-PRF and BCP has better outcome than BCP alone in management of periodontal defects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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