EFFECT OF PLATELET-RICH PLASMA IN COMBINATION WITH BONE ALLOGRAFT IN TREATMENT OF PERIODONTAL INTRA-BONY DEFECTS IN SMOKERS

Hossam A. Eid¹, Basma M. Zakí²*

1. Assistant Professor, Oral Medicine and Periodontology Department, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. Department of Periodontics, College of Dentistry, Gulf Medical University, Ajman, UAE.
2. Assistant Professor, Surgery and Oral Medicine Department, Oral and Dental Research Division, National Research Centre, Egypt.

Abstract

This study was conducted to evaluate the influence of smoking habit on the success of periodontal regeneration in treating intra-bony periodontal defects when using Platelet Rich Plasma (PRP) combined with Freeze Dried Bone Allograft (FDBA).

Thirty patients all diagnosed as having advanced chronic periodontitis were selected. The patients were equally allocated into two equal groups: Group I; (n=15) which included non-smoking patients having periodontal intra-bony defects managed with PRP and FDBA and Group II; (n=15) included smoking patients having periodontal intra-bony defects managed with PRP and FDBA. Clinical evaluation recording the plaque index (PI), gingival index (GI), pocket depth (PD), and clinical attachment level (CAL) was done. Radiographic assessment was also carried out to calculate the amount of bone gain. Estimation of the level of Platelet Derived Growth Factor (PDGF-BB) was performed.

The results revealed that the clinical improvement, the amount of bone gain and the level of PDGF-BB were more in the non-smokers group.

Smoking has a negative impact on the treatment outcomes of the periodontal intra-bony defects following the combined treatment of PRP and FDBA.

Keywords: Smoking, intra-bony periodontal defects, PRP, FDBA.


Introduction

The purpose of periodontal therapy is to arrest the destruction accompanied with the periodontal disease progression and to regenerate the lost structures achieving optimal comfort, function and esthetic appearance¹. The etiology of the chronic inflammatory periodontal disease process is multifactorial in nature. It results from the interaction between the microbial infection and the host defense finally leading to clinical attachment loss and alveolar bone resorption²-⁴.

Various studies conducted on animals and humans proved that smoking can damage the vascular and immunological systems tipping the balance favoring the tissue breakdown ⁵. It decreases the self-healing capacity of the tissues of the periodontium. It was documented that, the outcome of periodontal treatment in smokers is less favorable as the host cytokine levels in bio-fluids is affected in smokers ⁶. In comparison to non smokers, periodontal pathogenic bacteria conquer higher levels in smokers’ patients. This is caused by the direct modulation of the sub-gingival microflora favoring the colonization of these pathogenic bacteria ⁷.

In addition, there is increased evidence that smoking may interrupt the normal healing events of the periodontal tissues ⁸.

Clinical studies showed that after adjusting the plaque accumulation to be similar in both smokers and non-smokers the individuals...
who currently smoke presented deeper pockets probing depth, greater loss of attachment, more loss of bone, increased accumulation of supragingival calculus deposits and presence of less number of teeth. Revascularization which is mandatory for soft tissue healing and periodontal regeneration in periodontal surgeries outcome and implant placement can be negatively affected by smoking. The nicotine products in cigarettes can bind to root surface as reported in vitro studies, leading to alteration of fibroblast attachment and integrin expression with decrease in collagen production and increase in collagenase activity. Additionally, root surfaces of extracted teeth from smokers were having less periodontal ligament (PDL) fibroblast attachment when compared to those teeth from non-smokers.

Different types of bone grafts have been studied with different outcomes all aiming at regeneration of periodontal defects. Autogenous bone grafts are capable of providing viable osteogenic cells. The use of allografts produced either from humans from living donors or cadavers as a source of osteogenic cells. In addition they have the ability to act as space fillers maintaining room under guided tissue membranes. They can act as a vehicle for growth factors as well.

Platelet Rich Plasma (PRP) is a component of autologous plasma having a platelet concentration above baseline rich in platelet derived growth factors (PDGF) and transforming growth factors (TGF-ß). It is obtained by isolation and concentration of human platelets by gradient density centrifugation forming platelet gel.

It has been acknowledged that PRP can decrease the bleeding during and after the operations both at the donor and recipient sites due to their potential in healing acceleration. It can help in initially stabilizing the grafted tissue at the recipient sites due to its cohesive and adhesive nature as played in the wound healing process.

Deminerlized Freeze Dried Bone Allograft (DFDBA) has proven to show significant outcomes in the soft and hard clinical tissue parameters when used in treatment of intraosseous periodontal defects. The substances present in this grafting material i.e. the bone morphogenic proteins is capable of stimulating the local cells to produce new bone. The cell morphology and the chemical integrity are still maintained even after the cells were destroyed by the freezing and the drying process. The process of its manufacturing eliminates the concerns about the immunological responses and the possibility of transmitting disease and infection. Several studies reported the successful use of PRP together with various bone grafts for correcting periodontal osseous defects.

The goal of the current contemplate was to clinically and radiographically evaluate the treatment outcomes resulting from combining both PRP and DFDBA to correct periodontal intra-bone defects in smokers.

**Patients and Methods**

Thirty systemically healthy patients (18 males and 12 females), with an age range between 20-45 years (mean age 32.8 ± 1.25) diagnosed with advanced chronic periodontitis according to the classification of the American Academy of Periodontology 2000. These patients were selected from the outpatient’s clinics at the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt and the dental clinic in the medical services unit at the National Research Centre (NRC). The study protocol was approved from the Ethical Committee for Dental Studies at the Faculty of Dentistry, Suez Canal University, Ismailia, Egypt and the selected patients signed an informed consent form approving their participation after all the study procedures were explained to them. The selected patients have a total of forty intra-bony defects. Patients included in the study were systemically healthy with no contraindications to perform the surgical operation and are having normal platelet counts. They did not receive antibiotics in the past six months prior to treatment and no periodontal therapy in the past two years also. The patients should have at least one vertical osseous defect with probing pocket depth > 5mm. It should be a radiographic evidence of vertical/angular bone loss in the affected sites (at least one site in the quadrant) and had at least 2 mm of keratinized gingiva on the facial aspect of the selected tooth. Patients who are allergic or sensitive to any medications and / or local anesthesia used in the study were excluded from the study. Also pregnant and lactating mothers were not included in this contemplate. Teeth with
severe attrition and extensive mobility were excluded from the selection of the cases.

**Initial Periodontal Therapy**
Scaling and root planing (SRP) was performed for all the patients in addition to oral hygiene instructions teaching.

**Study Design**
All the thirty patients selected to be included in this experiment were equally divided into two groups: Group I; (n=15) which included non-smokers patients with periodontal intra-bony defects corrected with PRP and FDBA and Group II; (n=15) included smoking patients with periodontal intra-bony defects corrected with PRP and FDBA.

Periodontal assessment was carried out before starting the initial therapy (baseline) and at 6 and 9 months after treatment for both groups. At baseline evaluation, all clinical parameters were measured and mechanical treatment including removal of all supra and sub-gingival calcified deposits to obtain smooth and clean surfaces was done. For each patient the oral hygiene was checked every two weeks for 9 months. All patients were trained and encouraged to maintain their dental health and plaque control through brushing and flossing. All clinical assessment measurements were taken by one investigator. All subjects received clinical examination including recording of the following periodontal parameters for all the teeth; Plaque index (PI) 14, gingival index (GI) 15, pocket depth (PD) 16 and clinical attachment level (CAL). All the clinical measurements were done at six locations for all teeth named mesiobuccal, mesiolingual, midbuccal, distobuccal, distolingual, and midlingual. PI was assessed by measuring the presence or absence of supragingival biofilm with performing of a sweeping motion of the probe around the buccal, mesial, distal, and lingual sites of all teeth. Marginal gingival bleeding was estimated with GI. PD was recorded from the free-gingival margin to the base of the periodontal pocket (measured in millimeters) using UNC-15 periodontal probe and CAL was recorded from the cemento-enamel junction of the tooth to the base of the periodontal pocket (also recorded in millimeters). Utilizing the Michigan 0 probe with Williams' markings, all the measurements were approximated to the most elevated whole millimeter. The CEJ determination was made by using the anatomical CEJ or the apical extent of the existing restoration margin on each tooth. Plaque assessment using disclosing tablets were assessed every two weeks along the whole 9 months study period.

**Radiographic Assessment**
Intraoral periapical radiographs were taken using parallel technique with a Rinn XCP film holder (ENDEX INSTRUM, Germany). The distance from the alveolar crest (AC) 17 to the base of the bone defect depth was recorded. The point at which the periodontal ligament space is continuously in width at the most coronal point is considered the base of the defect (BOD). The difference between the initial and the final defect depth at the recall time intervals was considered as the amount of defect fill or bone gain occurring. Percentage of bone gain or defect fill was also calculated 11.

**Preoperative evaluation**
Occlusal stents were fabricated with cold cured acrylic resin on a cast model obtained from an alginate impression for accurate positioning of the periodontal probe aiming at reproducibility. The occlusal stent was made to cover the occlusal surface of the tooth being treated and the occlusal surfaces of at least one tooth in mesial and distal directions. Stents were also extended apically on the buccal and lingual surfaces to cover the coronal third of the teeth involved 6. Post-surgical measurements were done using the same probe position and angulation grooves prior to surgery. All smokers were advised to stop smoking at least for two months following the surgical procedures and were directed to smoking cessation program.

**PRP Preparation**
One hour before the surgery, 10 ml of blood was withdrawn from the antecubital vein of the patient and collected into vacutainer tube containing sodium citrate as an anticoagulant. It was then placed in an automated centrifuge. First spin of 1200 rpm for 20 min separated the whole blood into three fractions that is, platelet poor plasma (PPP), PRP and red blood cells (RBC). After discarding the RBC fraction a second spin of 2000 rpm for 15 min was given that separated the components into PPP and concentrated PRP (cPRP) 18. After discarding the PPP, the
remaining cPRP was activated by clot initiator (human thrombin) and 10% calcium chloride to obtain a sticky gel consistency. Platelet counts were performed on each sample, including a peripheral blood count and PRP count by hemocytometer. Minimum 3 times increase in platelet count (PC) from baseline PC were considered adequate for use.

Surgical procedures
The surgical procedure was performed by local infiltration of 2% xylcaine hydrochloride with adrenaline 1:80,000. Buccal and lingual/palatal sulcular incisions were used, and full-thickness mucoperiosteal flaps were elevated with a blunt dissection using a periosteal elevator. Care was excised to preserve as much inter-proximal tissue as possible. After meticulous defect debridement and removal of granulation tissue, grafting materials were placed up to the vertical height of the corresponding adjacent bone level. Surgical flap was repositioned and sutured with 3-0 black silk and covered with a periodontal pack.

Post-operative Care
Patients were given analgesics (ibuprofen 400 mg every 8 h) and antibiotics (amoxicillin 500 mg every 6 h) for 5 postoperative days and were advised to use 0.12% chlorhexidine mouth rinse twice daily for 14 days. Subjects were recalled after 1 week for suture removal, thereafter every two weeks for oral hygiene condition assessment during the whole experiment period.

Laboratory Investigations
GCF samples were gathered from the post-surgical sites after periodontal pack removal. Prior to GCF sampling, the supragingival plaque was evicted from the surfaces with a sterile curette carefully; these surfaces were desiccated with water spray, dried gently by an air syringe and isolated with cotton rolls. GCF was sampled using filter paper strips (Perio-paper, IDE Interstate, Amityville, NY, USA) carefully inserted into the crevice until mild resistance was felt and left there for 30 seconds. Care was taken to elude any possible mechanical injury. Strips contaminated with blood were discarded. Immediately, the volume of the sample was measured with the aid of a calibrated Periotron 8000 (Oraflow Inc., Amityville, NY, USA). After volume measurements, the strips were placed into sterile eppendorf tubes containing 300 µL PBS (Phosphate buffered saline). All GCF samples were immediately stored at −20°C until subsequent analysis. The readings from the Periotron 8000 were converted to an actual volume (µL) by reference to the standard curve. Later the samples were examined by Enzyme Linked Immuno-Sorbant Assay (ELISA) technique (Quantikine Kit, R&D systems, UK) to detect the concentrations of the Platelet Derived Growth Factor (PDGF-BB), for the groups included in this contemplate.

Statistical Analysis
Numerical data were presented as mean and standard deviation (SD) values. Data’s normality was explored using Kolmogorov-Smirnov test of normality. Mann Whitney U test was essential in comparing PI, GI, PD, and CAL before and after treatment in both studied groups at the different time periods. The percentage of change between baseline and different follow up times was explored in both groups using either Mann-Whitney or student t-test according to the normality of the data. Percentage of radio-opacity was calculated, which was analyzed by chi-square test. The significance level was set at \( p \leq 0.05 \). Statistical analysis was performed with SPSS 18.0, Chicago, IL, USA.

Results
The healing of soft tissue in all patients included in this contemplate was uneventful and lacked any inconvenience postoperatively during the follow up period of nine months. Only during the first week, analgesics were minimally used because the patients experienced moderate pain and discomfort. None of the patients showed post-operative swelling. After one week pain subsided completely.

1) Clinical Assessment:
Table (1) shows the mean ± standard deviation (SD) and \( p \)- values of PI, GI, PD and CAL before and after treatment in both groups I and II.

   a) Plaque index (PI) measurements:
The results revealed no statistical significant difference between Groups I and II before treatment with \( p \)-value 0.0262. After treatment there was a statistically significant decrease in PI scores in both groups after 6
months with \( p \)-value 0.059 and also after 9 months with \( p \)-value 0.023.

<table>
<thead>
<tr>
<th>Time</th>
<th>PI Group I (PRP+DFDBA)</th>
<th>PI Group II (PRP+DFDBA)</th>
<th>Mann-Whitney U test ( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.5 ± 0.42</td>
<td>2.9 ± 0.34</td>
<td>0.262</td>
</tr>
<tr>
<td>6 months</td>
<td>1.4 ± 0.43</td>
<td>1.8 ± 0.29</td>
<td>0.049*</td>
</tr>
<tr>
<td>9 months</td>
<td>1.2 ± 0.33</td>
<td>1.5 ± 0.50</td>
<td>0.023*</td>
</tr>
</tbody>
</table>

| GI Baseline | 2.5 ± 0.55 | 2.6 ± 0.53 | 0.061 |
| 6 months | 1.8 ± 0.44 | 1.3 ± 0.47 | 0.022* |
| 9 months | 0.4 ± 0.53 | 0.1 ± 0.24 | 0.021* |

| PD Baseline | 5.8 ± 0.88 | 5.6 ± 1.02 | 0.099 |
| 6 months | 3.3 ± 0.56 | 2.3 ± 0.66 | 0.017* |
| 9 months | 2.3 ± 0.40 | 1.5 ± 0.50 | 0.014* |

| CAL Baseline | 4.2 ± 0.77 | 4.1 ± 0.88 | 0.742 |
| 6 months | 3.4 ± 0.44 | 2.7 ± 0.51 | 0.023* |
| 9 months | 2.5 ± 0.52 | 1.4 ± 0.83 | 0.008* |

Table 1. Showing the mean ± SD values of PI, GI, PD and CAL at baseline, 6 and 9 months in both study groups

b) Gingival index (GI) measurements:
The results showed no statistically significant difference between groups I and II before treatment with \( p \)-value 0.661. Following treatment there was a statistically significant drop of GI scores in both groups after 6 months with \( p \)-value of 0.022 and after 9 months with \( p \)-value of 0.021.

c) Pocket depth (PD) measurements:
The results showed that there was no statistically significant difference between group I and II before treatment with \( p \)-value 0.696. After treatment there was a statistically significant reduction of PD values in both groups after 6 months with \( p \)-value of 0.017 and after 9 months with \( p \)-value of 0.014.

d) Clinical attachment level (CAL) measurements:
There was no statistically significant difference between group I and II before treatment with \( p \)-value 0.742. After treatment there was a statistically significant decrease of CAL measurements in both groups after 6 months with \( p \)-value of 0.023 and after 9 months with \( p \)-value of 0.008.

2) Radiographic Assessment
Standardized Periapical radiographs were taken using the parallel technique with Rinn film holders at baseline and during the follow up periods for each case. These films were scanned by Scanrom 4E scanner connected to IBM computer. The images were analyzed by software in the computer and the percentage of radio-opacity was calculated, which was analyzed by chi-square test. Qualitative assessment of periapical films showed that there was an increase in bone density in both study groups along the different study periods. These changes in the percentage of radio-opacity were statistically significant at the different time intervals as shown in table (2).

<table>
<thead>
<tr>
<th>Group</th>
<th>PI</th>
<th>GI</th>
<th>PD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>44</td>
<td>37.0</td>
<td>28</td>
<td>59</td>
</tr>
<tr>
<td>II</td>
<td>52</td>
<td>48.5</td>
<td>84</td>
<td>90.19</td>
</tr>
</tbody>
</table>

Table 2. Showing the percentages of improvement of the various measured clinical parameters in both studied groups.

Looking to the results of the mean bone gain; it was revealed that there was a statistically significant increase in both groups I and II when comparing the baseline with the 6 and 9 months records with the more bone gain in Group I. Meanwhile there was no statistically significant gain of bone comparing the 6 and 9 months records with the more bone gain in Group I.

The results revealed that at baseline there was no significant difference in the percentage of radio-opacity between group I (55%) and group II (52%). After 6 months there was a statistically significant increase in the percentage of radio-opacity between groups I (75.4%) and II (62.1%). After 9 months the percentage of radio-opacity showed a statistically significant increase between groups I (85.8%) and II (71.2%) as shown in table (3).
Table 3. Shows the amount of bone gain and the percentages of radio-opacities in both study groups.

**PDGF-BB Concentration:**

ELISA was used to measure the PDGF-BB concentrations in gingival crevicular fluid (GCF) in both study groups at 3, 5 and 7 days as shown in table (4). The highest level of PDGF concentration was found in group I at the 3rd day (1734 µg). Smokers group (II) showed a lowest concentration of PDGF (553.9 µg). At 5 days both groups still have a high concentration of PDGF when compared to the control (5531.74 µg). Group II which less level of PDGF concentration (425 µg) than the control group. At 7 days the only group that retained a high PDGF concentration level was that of group I. The value of PDGF concentration was (1361 µg) which was statistically more elevated than that of group II.

**Table 4. Mean Values of PDGF-BB concentration (µg) in both study groups at 3, 5, 7 days.**

Discussion

The main goal of treating diseased periodontium is to create an environment capable of maintaining optimum health, comfort and function. Different clinical investigations have compared the difference of responses of non-smokers and smokers toward the various types of periodontal disease treatment modalities outcomes including both surgical and nonsurgical therapies. The increased use of DFDBA currently is related to its proved osteoinductive properties. Different histological studies documented that it is more effective in inducing periodontal regeneration. The addition of growth factors can provide extra stimulus for optimum regeneration through modulation of cells residing within the periodontium.

PRP is a rich source for obtaining growth factors. PRP when added to hydroxyapatite, bovine porous bone mineral and barrier membrane has been used to treat periodontal defects with documented good outcomes. Our study revealed the significant improvement in the measured clinical parameters namely PI and GI scores, PD and CAL measurements (at 6 and 9 months). Calculation of the percentages of improvement showed that the results are more favorable in the non-smokers group. This is line with the explanation in a study presenting that such changes in index scores might be attributed to the maintenance of oral hygiene along the time intervals of the study. Various studies have shown similar improvements in plaque and gingival indices.

In different evaluations conducted to treat intrabony defects investigating the influence of smoking on the periodontal regenerative process revealed less decrease in PD and gain of CAL in smokers as compared to non-smokers patients. This was attributed primarily to the decrease in gingival inflammation and shrinkage of pocket wall after treatment. The study pointed out the detrimental effect of smoking on the periodontal regenerative process as. Growth factors (GF) abundantly exist in PRP. Bone regeneration is affected by some of the GF such as PDGF and TGF-B. Successful results were reported from various animal and human studies provided solid evidence that PRP is capable of enhancing the wound healing.
process and aids in the reconstruction of soft tissues specifically in conjunction with bone grafts.  

It was evident from the results of the present experiment that, using PRP in combination with bone allografts to treat the bony defects caused by the destructive process of periodontal disease, can effectively improve all of the tested clinical periodontal parameters. These findings strengthen forward other studies.  

The mechanism by which PRP aids the regenerative process of the periodontium is not completely understood. However, it has been discussed that PDGF, present in PRP affects mostly the osteoblastic cell proliferation process. In addition, PRP contains high fibrin content that provides a sticky nature working as a haemostatic and stabilizing agent that might aid in blood clotting and bone graft immobilization in the defect area, hence, plays an important role in periodontal regenerative procedures. PDGF proved to stimulate angiogenesis and differentiation of undifferentiated mesenchymal cells. It can also activate macrophages which play an essential role in secondary wound healing.  

It was also documented by Marx et al.  that adding concentrated platelets to bone grafts results in increased maturation rate and density of the autogenous grafts.  

It is noteworthy to mention that, there are some studies reporting that PRP preparation has a limited potential to promote local bone formation; using of PRP did not provide any significant outcome on bone implant contact, a limited bone formation was obtained with Bio-Oss with or without PDGF. No obvious positive effect on bone formation or working as osteoinductive material was reported when PRP was used after an implantation period of four weeks and even no negative effect was documented.  

Results of the present study seem to be not consistent with these findings. This contradiction can be attributed to several factors including: degree and severity of periodontal disease affecting included patients; concentration of PRP used; parameters used in assessment of periodontal status before and after treatment, time of evaluation during follow up as well as, methodological characteristics of each investigation. The assessment of periodontal condition of the patients included in the present study was performed using more than one clinical parameter, as dependence upon only one parameter can not reflect clearly the exact disease or health status.  

The absence of any allergic reactions, abscesses, or rejection of the implanted materials documented the high tolerance of the materials used. This is in line with previous studies proving the absence of allergic or foreign body reactions.  

Results of this contemplate reported the high bone gain which is more obvious in the non-smokers group denoting the detrimental effect of smoking on the periodontal regenerative process.  

A study was conducted in non-smokers reporting the high amount of bone gain and the increased percentage of bone fill in sites managed with the combination of PRP and ß-tricalcium phosphate (ß-TCP). Also other contemplate adding PRP to bone grafts to treat intrabony defects presented the beneficial effect of this combination by the increased radiographic maturation rates measured.  

In line with our study greater bone height was observed when PRP was used with allogenic bone in treating periodontal bony defects. The same results were obtained after using hydroxyapatite with PRP showing the significant defect fill.  

On the other hand non-significant difference was revealed in a contemplate by Tamura et al examining the relative amounts of mineralized bone when PRP was added to porous ß-TCP in treating intra-bony defects.  

Other studies conveyed an unfavorable outcome after periodontal regeneration in smokers having different types of periodontal defects. Even though, the exact mechanism of how smoking affects the periodontal regeneration outcome is not clarified yet. Some in vitro studies conveyed that the proliferation, attraction and chemotaxis of periodontal ligament cells is adversely affected by nicotine and smoking by products. Not only this, but also the latter intensifies the effect of periodontal pathogenic toxins.  

Moreover, smokers revealed a deteriorated peripheral blood supply owing to vasoconstriction caused by nicotine and debilitating oxygen transport and metabolism caused by carbon monoxide. Therefore, since smoking seems to interrupt stages of the reparatory/regenerative process in
the periodontal wound it can thereby compromise generally the healing process.

Conclusion

The results of the present contemplate documented the negative effect of smoking that can impair the healing outcomes of periodontal regeneration.

Declaration of Interest

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