Comparative Evaluation of Injectable Platelet Rich Fibrin (I-PRF) with DFDBA and Titatium Prepared Platelet Rich Fibrin (T-PRF) with DFDBA In The Treatment of Infrabony Defects In Chronic Periodontitis Patients - A Clinico-Radiographical Study

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Abstract
Objectives: The main objective of this study was to evaluate the efficacy of Titanium prepared platelet-rich Fibrin (T-PRF) and Injectable Platelet Rich Fibrin (I-PRF) with demineralized freeze-dried bone allograft (DFDBA) in order to improve the clinical and radiographic results obtained in treatment of periodontal infrabony defects.

Materials and Methods: This split-mouth randomized controlled clinical trial was conducted on 30 subjects with two comparable bilateral infrabony defects. Plaque Index (PI), Gingival Index (GI), Clinical attachment level (CAL), Periodontal Pocket depth (PD), as well as radiographic parameters IOPA including the radiographic defect size, were measured at six months post-operatively.

Results: The mean reductions in PD and CAL were 4.0±0.77 mm and 2.5±0.68 mm in sites treated with I-PRF+DFDBA, respectively (P<0.05); these reductions were 4.2±0.86 mm and 3.1±0.62 mm, respectively in T-PRF+DFDBA group (P<0.05). Radiographic evaluation revealed reduction in the radiographic defect size in the I-PRF+DFDBA and T-PRF+DFDBA sites. Statistically, there were no significant differences between the two treatment modalities.

Conclusion: This study showed that both treatments resulted in significant improvement in the probing depth reduction, clinical attachment level gain and radiographic size of the infrabony defect at six months after surgery. I-PRF with DFDBA membrane in treatment of infrabony osseous defect showed more defect fill followed by T-PRF along with DFDBA membrane.

Keywords: Chronic periodontitis, Infrabony defects, Injectable Platelet rich fibrin(I-PRF), Titanium Platelet rich fibrin(T-PRF), Demineralized Freeze Dried Bone Allograft(DFDBA), Cone-Beam Computed Tomography(CBCT)

Introduction

Periodontitis is non-reversible inflammatory disease occurs as a result of imbalance between immune response of host and dysbiotic plaque biofilm that leads to change in the normal architecture of the alveolar bone and other supporting periodontal apparatus. It increases the risk of substantial proportion of edentulism and masticatory dysfunction that results in significant dental care costs and has a negative impact on general health. Thus, treatment of this condition is necessary.
Although the host response, anatomical factors and tissue structural factors are important in determining the extent and severity of periodontal disease. Severe periodontitis results in progressive attachment loss and it is sixth most prevalent disease in human being. Alteration in form and function of alveolar bone due to periodontitis leads to bone defects.

The most extensively evaluated graft material used for the treatment of infrabony defects are allograft, Decalcified Freeze-Dried Bone Allograft (DFDBA), due to additional demineralization step it induced bone formation when exposed to bone morphogenetic proteins and are composed of acidic polypeptides.

The second generation of platelet concentrate known as PRF was developed by Choukroun et al. in France in 2001 as an autologous biomaterial that contains leucocytes and PRF.

PRF does not require any anticoagulation or gelling agent for its preparation, thus making the PRF protocol easier to collect a fibrin clot by polymerization process through centrifugation, producing natural fibrin architecture responsible for slow release of growth factors in \( > 7 \) days. The injectable formulation of PRF (I-PRF) can be utilized easily with bone graft (DFDBA). By taking advantage of this slower and shorter centrifugation speeds, a higher presence of regenerative cells with higher concentrations of growth factors can be observed. Growth factors are used to enhance the efficacy of periodontal regeneration. PRF is the most effective because it is derived from the patient's own blood. No risk of infection transmission and absence of immunological reactions are among the advantages of PRF.

The platelet concentrate evolved by changing the structural design tube, using a biocompatible material titanium known as Titanium prepared Platelet Rich Fibrin. T-PRF is a second generation platelet concentrate developed to overcome the hazardous effect of silica in the glass vacutainers used for the preparation of (PRF) Platelet Rich Fibrin and it has also showed more biocompatibility lead to polymerized fibrin formation with a longer resorption rate in the tissues.

Successful clinical result has been reported with PRF but possible health hazards due to the use of glass vacutainer collection tubes for the blood with silica activators, although dense enough to sediment with the red blood cells, are sufficiently small for a fraction to remain in colloidal suspension in the buffy coat, fibrin, and platelet-poor plasma layers and was thus contaminate any therapeutic application to the patient. The injectable formulation of PRF (I-PRF) to overcome the hazardous effect and can be utilized easily with bone graft (DFDBA). By taking advantage of this slower and shorter centrifugation speeds, a higher presence of regenerative cells with higher concentrations of growth factors can be observed.

Therefore, the aim of this randomized controlled bilateral infrabony defect prospective clinical and radiographic trial was to compare the extent of periodontal regeneration with the use of I-PRF in combination of DFDBA and T-PRF with DFDBA.

Materials and Methods

This controlled, split mouth clinical trial, approved by the Institutional Ethics Committee of D.J. DENTAL Ethics Committee, D.J. College of Dental sciences and Research, comprised of 30 participants (age range, 18-55 years) with bilaterally similar periodontal infrabony defects. Participants were selected from the outpatient department from D. J. College of Dental Sciences and Research, Modinagar. The participants agreed to participate in the study and gave their written informed consent. Participants with a probing depth (PD) maximum 9 mm or less than 9 mm without mobility at two or more sites, sites exhibiting clinical and radiographic evidence of infrabony defects, and two or three wall infrabony defects at two sites were included in the study. Participants on chemotherapy and radiotherapy, Alcohol Abuse, Systemic
disorder (uncontrolled diabetes, autoimmune disease, etc), with bone disorder, pregnant and lactating women, participant under anticoagulation treatment, bleeding disorder, with allergy to medication; those unable to maintain meticulous oral hygiene after Phase I therapy were excluded from the study.

Study design and group allocation

Group I: Patient with intrabony defects were treated with I-PRF + DFDBA (30 sites)

Group II: Patient with intrabony defects were treated with T-PRF Membrane + DFDBA (30 sites)

Obtaining Platelet-Rich Fibrin

I-PRF Preparation

To obtain the I-PRF, blood collection will be performed using 10 ml sterile tubes without any additive (anti-coagulant) then centrifuge at 700 rpm at 3-4 min at room temperature. The upper liquid layer will be collected as I-PRF.

T-PRF Preparation

Just prior to the surgery, intravenous blood collected in a 10 ml sterile titanium test tube without anticoagulant by venipuncturing of antecubital vein. Tube will be immediately centrifuged at 2700 rpm 12 min in a centrifuge machine. Blood centrifugation immediately after collection allows the composition of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (platelet poor plasma (PPP)) at the top. T-PRF clot thus formed is then separated using sterile tweezers and scissors and a stable fibrin membrane will be obtained by squeezing serum out of the T-PRF clot. Apart of membrane was minced to be used as graft material and another part was trimmed as membrane to cover the defect.
Figure: Titanium tube

Group-I

At Baseline

DFDBA graft with 1-pre
graft placed in infrabony defect

Reflection of Flap and Degranulation of site

Post-operative suture placed

Group-II

At three months

At six months

Figure: T-PRF clot
GROUP - II

AT BASELINE

REJECTION OF FLAP AND DEGRANULATION OF TISSUE

DIDIBA GRAFT AND T-PLATELET RICH FIBRIN PLACEMENT

SUTURES PlACED

RADIOGRAPHIC ANALYSIS

Group - I

At Baseline

At Three month

At six month

RADIOGRAPHIC ANALYSIS

EXPERIMENT GROUP II

AT BASELINE

AT THREE MONTH

AT SIX MONTH
The changes in PI, GI, PD and CAL and radiographic IOPA and CBCT changes were analyzed at baseline and postsurgically after 3 months and 6 months in each group with Mean or Average, Standard Deviation, paired t-test and between the two groups with unpaired t-test. 

**Demineralized Freeze Dried Bone Allograft:**

The material was procured from Tata Memorial Hospital Mumbai and is available in the size of 125 micron to 1000 micron. In this study size of 125-500 micron was used. The particle size of the DFDBA is an important variable in the success of DFDBA as a bone inductive material particle size from 125 to 1000 micron possesses higher osteogenic potential then do particles below 125 microns.

**Radiographic examinations:**

During the first phase of treatment, at baseline, CBCT was obtained from the surgical regions and postoperatively at six months. IOPA obtained at baseline, at 3 months and at 6 months. The CBCT scans obtained were 3D evaluation of cross sectional and coronal images helped us determine the defects size in all directions.

**Surgical Procedure**

Povidone iodine was used to wiped extraorally.
(5%) and in order to achieve adequate local anesthesia (2% lidocaine HCl with adrenaline 1:80,000) was instituted before initiating the surgical procedure. Buccal and lingual/ palatal sulcular incisions were given using BP blade No.12/15 and handle No.3 at the tooth of interest, one tooth mesially and another distally. Papilla preservation flap incisions were given wherever required. Full thickness mucoperiosteal flaps were elevated by means of blunt dissection using a periosteal elevator to expose the defect. Care was taken to preserve as much interproximal tissue as possible. A thorough debridement using Hu- friedy Universal and Gracey curettes was carried out in the defect area. All the granulation tissue was removed to ensure a clean site followed by thorough root planing. The minced T-PRF and I-PRF will be filled into the intrabony defect and another part of it was adapted over the grafted defect. The flap will repositioned to their presurgical levels and sutured with 3-0 non-absorbable silk sutures ultitized an interrupted followed by placement of periodontal pack. Both the treatments will be performed with the same surgical protocol as above mentioned.

**Post-Operative Instructions**

These were given to all patients including first 24 hours after the operation, patients were advised to avoid hot foods and liquids to permit the pack to harden and to avoid chewing from the operated side. Liquids and semi-solid or finely minced cold foods were suggested. Patients were asked to take the prescribed medication following the surgical procedure. Patients were advised to avoid brushing the teeth in the surgical area. Chlorhexidine digluconate mouthwash (0.2%) was advocated twice daily with a dilution of 1:1 (mouthwash: water) for 2 weeks. Patients were advised warm saline rinses 24 hours post-surgery. They were also asked not to feel the surgical area with the tongue or finger. In case of any problem, they were asked to report to the clinic the next day.

**Results**

After the phase I therapy, thirty patients with 60 sites were selected based on inclusion and exclusion criteria. Out of the 30 patients selected, 4 patient 8 sites fail to maintain the plaque score and gingival score of equal to or less than one and thus were excluded from the study. Fifty two sites were selected from twenty six systemically healthy individuals (11 males and 15 females) after fulfilling the inclusion criteria. The sites were allocated according to slip system. In the group I nine sites where in the maxillary arch and seventeen out of twenty six sites were in the mandibular arch similarly in the group II same nine sites were in maxillary arch and seventeen were in mandibular arch.

All the sites were clinically evaluated at baseline, three months and six months post-surgery. In all the patients healing of the defects was uneventful and no post-operative complications arose in any of the patients thus indicating the biocompatible nature of the material. During the course of treatment, plaque index had no change while bleeding on probing. The mean change in plaque index when compared between experimental group I and experimental group II decreased from baseline (0.001±0.01) to three month (-3.45±0.32) and then increased to 6 months (0.008±0.29) post surgery. However, no statistical difference was found between the groups at baseline (p=0.6541), three months (p=0.6522) and six months (p=0.6123). Table 1

The mean change in gingival index when compared between two groups from baseline (-0.014±0.16) to three month (-0.08±0.12) and further decreased to 6 months (-0.13±0.05) post surgery. However, no statistical difference was found between the groups at baseline (p=0.7310), three months (p=0.5461) and six months (p=0.7893). Table 2

The Pocket Depth (PD) was calculated by subtracting the distance from Fixed Reference Point to Base of the Pocket and Fixed reference point to Gingival Margin. In group I, the mean PD recorded at baseline was 6.2 with a standard deviation (SD) of 0.98. It decreased to
4.2±0.87 at the end of three month post treatment and further decreased to 2.5±0.68 after a period of six months. The mean difference from baseline to three months was 2.3 with a standard deviation of 0.87 which was statistically significant (p=0.0002) and 4.0 with a standard deviation of 0.77 post six months, which was statistically significant (p=0.0012). Also the mean difference from three months to six months was 1.8±0.70 which was statistically significant (p = 0.0002). In group II, the mean PD recorded at baseline was 6.4 with a standard deviation (SD) of 0.88. It decreased to 3.8±0.84 at the end of three month post treatment and further decreased to 1.8±0.92 after a period of six months. The mean difference from baseline to three months was 2.8 with a standard deviation of 1.3 which was statistically significant (p=0.0001) and 4.2 with a standard deviation of 0.86 post six months, which was statistically significant (p=0.0000). Also the mean difference from three months to six months was 1.9±0.98 which was also statistically significant (p= 0.0000).

The mean change in PD when compared between the groups were increased from baseline (-0.1±1.58) to three month (0.4±1.25) and further increased to 6 months (0.6±1.34) post surgery in group II. However, no statistical difference was found between the groups at baseline (p=0.7302), three months (p=0.6013) and six months (p= 0.5514).table 3

The Clinical Attachment Level (CAL) was calculated by difference between Fixed Reference Point to Base of the Pocket and Fixed Reference Point to Cemento Enamel Junction. The mean change in CAL when compared between the groups were decreased from baseline (55.77±123.88) to three month (65.66±142.82) and decreased to 6 months (26.01±86.41) post surgery. However, no statistical difference was found between the groups at baseline (p=0.0002), three months (p=0.0001) and six months (p= 0.0002).

**Discussion**

There are always controversies about the effect of PRF or PRF used in combination with other graft materials. This study was designed to compare the efficacy of I-PRF with DFDBA and T prepared PRF with DFDBA. Although there was no significant difference between the two treatment modalities, significant improvement was noticed in PD reduction, clinical attachment gain and infrabony defect fill.

In previous study done by Carranza FA et al stated that the management of periodontal osseous defects, including destruction of the periodontal ligament, cementum and the formation of infrabony defects, has always been a challenge in clinical periodontics. During the 1950s and into the 1960s, resective surgical therapy with or without osseous recontouring was considered the norm in the belief that attainment of shallow pocket depths was a worthwhile goal. Since then attention has been focused more on regenerative and reconstructive therapies rather than resective therapies.

PRF is an autologous concentration of platelets in plasma. It is a second generation platelet concentrate widely used to accelerate soft and hard tissue healing. The PRF in the present study was prepared in accordance with the protocol developed by Choukroun et al in 2001. The processing of PRF is a simple and inexpensive technique, and the systematic use of this biomaterial for periodontal regeneration seems a very promising option. Further presence of fibrin network facilitates cellular migration, vascularization, and survival of the graft, the growth factors such as (PDGF, TGF-β, IGF-1) are gradually released as the fibrin matrix is resorbed, thus creating a perpetual process of healing. The
presence of leukocytes and cytokines in the fibrin network play an important role in the self-regulation of inflammatory and infectious phenomena within the grafted material thus facilitating regeneration. The practical aspect of PRF use in periodontal osseous defects may be clinically relevant too. Because PRF preparation utilizes the patient’s own blood, the risk of human to human/animal disease transmission is virtually eliminated. The present clinical trial demonstrates that the use of I-PRF and T-PRF membrane along with DFDBA in periodontal osseous defects, is an effective modality in promoting clinical resolution of Infrabony defects.6

A wide array of new materials has been used for promoting periodontal regeneration in intraosseous defects. The bone replacement grafts provide regeneration through inductive or conductive processes and in combination with growth factors, have the potential to optimize the outcome of periodontal regeneration. Sunitha J stated that Proliferation and migration of periodontal ligament cells and synthesis of extracellular matrix as well as differentiation of cementoblasts and osteoblasts is a prerequisite for obtaining periodontal regeneration and growth factors may represent a potential aid in attempts to regenerate the periodontium.17 This study combined the effectiveness of DFDBA with Injectable-Platelet Rich Fibrin and with Titanium-Platelet Rich Fibrin to enhance the regenerative potential of the graft.

The observation in the the study of Miron et al7 concluded that I-PRF can release higher concentrations of various growth factors and induce higher fibroblast migration and expression of PDGF, TGF-β, and collagen1. I-PRF also showed significantly higher levels of total long-term release of these factors. I-PRF application can be more feasible and minimally invasive without using any additives for its preparation.

In this present study, it was observed that I-PRF had highest number of platelet count and it was statistically significant. This could be attributed to the low centrifugation speed and time resulting in their higher number. PRP although being an autologous preparation requires the addition of thrombin and calcium for its activation. These additives can result in the development of antibodies to the clotting factors V, XI, and thrombin, thereby adversely affecting the coagulation process and also can trigger an immune reaction. PRF itself contains physiologically available thrombin that is responsible for slow polymerization of fibrinogen into fibrin resulting in a physiologic architecture favorable to wound healing. This fibrin network protects the growth factors from proteolysis. Besides, PRF also favors the development of microvascularization leading to a more efficient cell migration. I-PRF was introduced based on the similar concept that of PRF with added advantage of it being in injectable form. This injectable form of PRF can be utilized alone or combined easily with various biomaterials. Its protocol is based on the concept that slower and shorter centrifugation spin results in a higher presence of regenerative cells with higher concentrations of growth factors. In a recently conducted study, it was observed that I-PRF formed a small clot as a result of fibrin components that acted as a dynamic gel with cells likely contained within its hydrogel. It was therefore hypothesized that an additional release of growth factors from I-PRF with DFDBA are more effective and can be excepted beyond 10 days. Ghanaati et al. introduced the “low-speed concept” for blood centrifugation whereby lower centrifugation speeds were shown to contain higher numbers of cells including leukocytes before the formation of a fibrin clot8

According to Dohan Ehrenfest’s classification, which defines four major categories for these products. Platelet-rich products can be classified as follows according to their leukocyte and fibrin content: only platelet-rich plasma products, platelet- and leukocyte- rich plasma products, only platelet-rich fibrin, and platelet- and leukocyte-rich fibrin. Dohan Ehrenfest et al9 stated that L-PRF method yields a second generation platelet- rich product because no
The cell composition of L-PRF implies that this biomaterial is a blood-derived living tissue that must be handled carefully to keep its cellular content alive and stable. T-PRF method is potentially beneficial if a titanium tube is used instead of a glass tube in the classical L-PRF method. T-PRF is a platelet- and leukocyte-rich fibrin similar to that obtained from the classical L-PRF method. Although T-PRF and the L-PRF methods are quite similar, the titanium-induced platelet activation provides distinctive characteristics to T-PRF. Titanium has one of the highest strength-to-weight ratios and corrosion resistance among metals. Due to its noncorrosive properties, titanium has excellent biocompatibility. The material passivates itself in vivo by forming an adhesive oxide layer. Titanium also displays a unique property of osseointegration, connecting both structurally and functionally with the underlying bone. Hemocompatibility is a key property for biomaterials that come into contact with the blood. The basic histological structure of T-PRF is similar to L-PRF; however, the fibrin of T-PRF seemed more tightly woven and thicker than that of the classic L-PRF. This difference may be due to a better hemocompatibility of titanium compared to glass, which could have potentially led to the formation of a more polymerized fibrin. Due to this structure, T-PRF may last a bit longer in the tissue.

In light microscopy, T-PRF appears denser in both fibrin network and cellular components as compared to PRF. In scanning electron microscopy, T-PRF appears to show denser fibers and denser infiltration of cells indicating the presence of dense fibrin matrix. The histologic picture is in accordance with Tunali et al. 4

The present study was a randomized, parallel, clinical trial and was carried out to assess the efficacy of DFDBA with I-PRF compare to DFDBA with Tinanium prepared PRF membrane(T-PRF) in the treatment of periodontal infrabony defects. The subjects were randomly assigned to, DFDBA with I-PRF and DFDBA with T-PRF Membrane. Parallel trials assures that any difference between treatments is in fact due to treatment effects (or random chance), rather than some systematic differences between the groups of subjects. Other advantages of parallel design are that it is simple, has a valid comparison, universally accepted, analysis is less complicated, interpretation of the results is straightforward. Parallel group design provides a more accurate and precise assessment of treatment.

A study done by Karde, et al. 10 the platelet count of I-PRF was statistically significant ($P < 0.001$). This indicates that I-PRF with DFDBA has maximum number of platelets in comparison to T-PRF with DFDBA. Also, when intergroup comparison at three month and six month was done the results were statistically not significant.

From the observations of Blaggana V 11 and Agarwal A 12 who suggested that osteoinductive property is because of the exposure of the BMPs and growth factors following the acid demineralization process of the allograft. These BMPs and growth factors permit rapid revascularization and hard tissue ingrowth in the osseous defects thereby promoting periodontal regeneration.

PRF was used as a GTR membrane along with DFDBA in the present study for the prevention of migration of junctional epithelium and as demonstrated by Alghamdi H, 13 it further separated and stimulated the interface between the gingival tissue and the root surface on the entire height of the flap thus acting as a healing and interposition biomaterial. This is in contrast to the study of Bansal C 14 and Khattar S 15 who evaluated PRF fragments and suggested that fragments serves as a biological connectors between bone particles.

According to the study done by Mitra, et al. 16 who suggested that T-PRF shows superior acceptability with bone grafts by demonstrating excellent containment of the material and resorbs without the formation of voids and detritus.
However more longitudinal clinical trials with larger sample size are required to compare the result of the T-PRF with DFDBA and I-PRF along with DFDBA. When DFDBA is combined with PRF membrane, controversial results exist in the literature regarding the osteoinductive role of BMP in bone replacement grafting materials. It can be speculated that because BMPs are members of the TGF superfamily, their effect, if BMPs exist in DFDBA and are active add to the effects of the growth factors within the platelets, ensuring a synergetic impact on the cell population of the wound as observed in present study. The gain in CAL and reduction in PPD in the present study made it safe to speculate that the absorption of I-PRF was slow enough to produce the desired effect. Logically, histologic studies are required to characterize the process of bioresorption of I PRF with DFDBA and T-PRF along with DFDBA.

In both the groups, the clinical attachment level gain was accomplished by significant reduction in the size of the defect. Thus clinical and radiograph result were well correlated. However this correlation should be evaluated with caution. Although some clinical studies have attributed the gain in clinical attachment to the formation of the junctional epithelium, only histological evaluation would be able to determine the type of attachment in the study. Nevertheless as no relation was established in this study between the characteristics of the defect observed intrasurgically it is not possible to determine its influence on clinical results.

Table 3: Intergroup comparison of difference in differences of mean values in Pocket depth between Experiment group I and Experiment group II at different time intervals

| TIME INTERVAL | EXPERIMENTAL 1 Vs EXPERIMENTAL GROUP II | t value | p value |
|---------------|-----------------------------------------|---------|---------|
| BL            | -0.1±1.58                               | 0.7302  |         |
| 3 M           | 0.4±1.25                                | 1.03    | 0.6013  |
| 6 M           | 0.6±1.34                                | 1.61    | 0.5514  |
| 3M- 6M        | -0.1±1.02                               | 1.26    | 0.4121  |

Table 4: Intergroup comparison of difference in difference of mean values of Clinical Attachment level between Experiment group I and Experiment group II at different time intervals

| TIME INTERVAL | EXPERIMENTAL 1 Vs EXPERIMENTAL GROUP II | t value | p value |
|---------------|-----------------------------------------|---------|---------|
| BL            | -0.4±1.51                               | 1.035   | 0.4420  |
| 3 M           | -0.1±1.2                                | 1.75    | 0.2368  |
| 6 M           | 0.2±1.31                                | 1.26    | 0.4121  |
| 3M- 6M        | -0.10±.75                               |         |         |

Summary

The results are summarized as no adverse reaction or allergy had been reported in both group throughout the study. There was a statistically significant reduction in probing depth in both the groups individually; however, on comparing the both group, the net reduction was not significant. There was a statistically significant gain in the clinical attachment in both groups individually; however, on comparing both groups, the resultant gain was not significant. Radiographic assessment, showed a decrease in the defect size in both groups.

Conclusion
Both the treatment modalities demonstrated a significant improvement in the probing depth reduction, clinical attachment level gain and radiographic size of the infrabony defect at six months after surgery. I-PRF with DFDBA group membrane in treatment of intrabony osseous defect showed more defect fill followed by T-PRF along with DFDBA membrane group. Long term analysis could provide better results. The sample size was limited and a larger sample size would have probably given more meaningful results. Histological examination was not feasible in the present human study, otherwise the results would have been more confirmatory.

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