Original

A Randomized Controlled Clinical Study of Autologous Platelet Rich Fibrin (PRF) in Combination with HA and Beta-TCP or HA and Beta-TCP Alone for Treatment of Furcation Defects

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Abstract: An increased knowledge of specific cellular response and function has led to the development of numerous treatment modalities based on the utilization of growth factors. The present controlled clinical study was undertaken to evaluate the effectiveness of autologous platelet rich fibrin (PRF) in combination with HA and beta-TCP in treatment of human class II furcation defects and to compare it with HA and beta-TCP alone. A total of 24 interproximal defects in 24 chronic periodontitis patients were included in the study. The test group was treated by an open flap debridement in combination with autologous platelet rich fibrin (PRF) in combination with hydroxyapatite beta tricalcium phosphate, while the control group was treated by an open flap debridement (OFD) along with hydroxyapatite and beta tricalcium phosphate. At 12 months, both the test and control groups showed significant mean PPD reduction and CAL gain. There was statistically significant (p<0.05) greater probing depth reduction of 1.50 mm for the test group compared to the control. The mean Clinical Attachment Level (CAL) gains of 3.0 ± 0.95 mm was observed in the test group, while the control group displayed mean CAL gains of 2.00 ± 0.85 mm. The observed differences between baseline CAL and 12 months CAL were found to be statistically significant in both the groups (p<0.05). The mean CAL gain observed in the test group was significantly greater than the control group. Horizontal probing depth were significantly reduced in test group (3.33 ± 0.83 mm) compared to control group (1.75 ± 1.21 mm). Frequency analysis of furcation changes revealed complete furcation closure in 50% sites in test groups than control group which showed only 16.66% sites of complete resolution of furcation defects. The treatment with PRF in combination with HA and β-TCP group resulted in a significantly higher CAL gain, PPD and HPD reduction in comparison with hydroxyapatite and beta tricalcium phosphate.

Key words: Platelet rich fibrin, Furcation, Beta tricalcium phosphate, Hydroxyapatite

Introduction

The ideal goal of furcation therapy is to retain the tooth by achieving complete closure of the furcation defect thus improving the prognosis of involved tooth1,2,3. Class II Furcation defects with their unique anatomy, pose a special therapeutic challenge. Several techniques have been proposed to treat and improve the clinical condition of mandibular class II furcation involved molars with varying results1,2,3. It was suggested that the combination treatment including both bone replacement graft + GTR would provide the most beneficial regenerative therapy for class II furcation defects4. The advantage of using a bone graft with membrane was to establish initial blood clot stabilization and decrease the potential of having dead space under the membrane5. However, the results obtained in controlled clinical studies demonstrated that the use of bone graft together with barrier membrane was of limited significance1,4,5. Therefore, the use of replacement graft to improve the result of guided tissue regeneration therapy was not clearly justified.

The emergence of different biomaterials such as the use of bone morphogenic protein (BMP), Enamel matrix protein derivatives (EMD) and growth factors in the field of periodontics have provided researchers with varied treatment options for the management of class II furcation defects. Studies have demonstrated that application of biomaterials in combination with bone graft substitutes were found to be an effective treatment modality in the management of infrabony defects and grade II furcation defects, exhibiting improvement in clinical parameters and bone fill5. However, there is no single regenerative material to be considered as the gold standard in the treatment of Grade II furcation defects.

Carroll et al.6 in his in vitro study demonstrated that the viable platelets in PRF releases growth factors like Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor (TGF), Insulin like Growth Factor (IGF), Epithelial...
Growth Factor (EGF) and basic Fibroblast Growth Factor (bFGF) in about the same concentration for a duration of 7 days. Beneficial effects of PRF have been studied in various surgical procedures like sinus floor augmentation during implant placement\(^9\) in multiple gingival recessions with coronally displaced flap\(^10\) and in facial plastic surgical procedures\(^11\). Beta tricalcium phosphate (β-TCP) is a purified, multicrystaline, porous form of calcium phosphate with Ca:PO\(_4\) ratio similar to that of natural bone material. It provides matrix or scaffolding for periodontal regeneration and also facilitates the stabilization of the blood clot\(^12\).

Recently Lekovic et al.\(^13\) demonstrated that, PRF in combination with bovine porous bone mineral has the ability to increase the regenerative effect in infrabony defects. The intended role of PRF in the infrabony defects was to deliver the growth factors in the early phase of healing. However, to our knowledge, there are no studies reported on the use of autologous PRF, in combination with bone graft, in the treatment of furcation defects. The aim of the present study was to evaluate the effectiveness of autologous PRF in combination with β-TCP in the treatment of human mandibular class II furcation defects.

**Materials and Methods**

A total of 24 class II furcation defects in 24 moderate to advanced chronic periodontitis patients in the age range of 34 to 49 years (40 ± 4.29) were selected for the study from the Outpatient Department of Periodontics, S P Dental College, Sawangi (Meghe), Wardha, India. Ethical approval was obtained from Institutional ethical committee vide reference number IEC/2589/2017. The patients were required to fulfill...
the following inclusion criteria: i) systemically healthy subjects, ii) Presence of Class II furcation defect involving either buccal or lingual surfaces of the mandibular molars as determined by clinical and radiographic evaluation, iii) Presence of <3 mm horizontal furcation probing depth, iv) Presence of <3 mm of vertical furcation probing depth, v) The experimental tooth having proximal bone height coronal to the inter-radicular bone level. Aggressive periodontitis patients and patients who are non-compliant to periodontal maintenance program (plaque index score >1), smokers, pregnant females or lactating mothers were excluded from the study.

After proper examination and diagnosis, initial therapy consisting of oral hygiene instructions, supra and subgingival scaling and root planing under local anesthesia were performed. Occlusal adjustment, if necessary, was performed to control occlusal trauma. A custom made occlusal acrylic stent was used to standardize the probing measurements.

A total of 24 class–II furcation defects in 24 patients on mandibular molars involving either buccal or lingual surfaces were found suitable after initial therapy. Prior to surgery, selected defects were randomly assigned to test and control groups by a coin flip each consisting of 12 defects, according to randomized parallel design. Test group was treated by an open flap debridement (OFD) along with PRF + HA and β-TCP (Ossift, Equinox Medical Technologies B.V, Amersfoort, Netherlands) and control group was treated by an OFD along with HA and β-TCP only.

The clinical measurements recorded were probing pocket depth (PPD), relative clinical attachment level (R-CAL), relative gingival marginal level (R-GML) and Horizontal probing depth (HPD) of furcation. All the clinical measurements were recorded on the day of surgery and at 12 months’ post-surgery. A blinded examiner made the pre-treatment and post treatment clinical measurements. Patient’s oral hygiene status was evaluated by plaque index. Gingival inflammation was assessed by papillary bleeding index.

**Surgical procedure (Fig.1A-L)**

The surgical procedure included a pre-surgical rinse, administration of local anesthesia (2% Xylocaine containing 1:80,000 concentration of epinephrine) and raising a full thickness mucoperiosteal flap on either of local anesthesia (2% Xylocaine containing 1:80,000 concentration of epinephrine) and raising a full thickness mucoperiosteal flap on either side of the defect. The root surfaces were planed until a smooth hard consistency was obtained. At this stage, horizontal defect depth (HDD) of the furcation defect was measured horizontally at its deepest location, using UNC 15 probe and another reference probe placed across the most prominent root surface to bridge the first probe. Vertical defect depth (VDD): of the furcation defect was measured vertically at its deepest location using the fornix of the furcation as the fixed reference point. The test site defect was treated with OFD with insertion of PRF + HA and β-TCP (Ossift, Equinox Medical Technologies B.V, Amersfoort, Netherlands).

**PRF preparation**

On the day of surgery, 10 ml of blood was drawn from each patient by venipuncture of the antecubital vein. Blood was collected in a sterile glass test tube (10 ml) without any anti-coagulant. Immediately test tube was centrifuged using a refrigerated centrifugal machine at 400 g for 12 min. Because of differential densities, it resulted in the separation of three basic fractions: a base of red blood cells at the bottom, acellular plasma on the surface, and finally a PRF clot between the two. A total of 2–3 ml of the top layer was pipetted out with the sterile dropper; the middle layer (PRF) was removed and placed in a sterile dappen dish.

One part of PRF along with beta-tricalcium phosphate (β-TCP) was placed in the furcation defect. The other part of PRF after preparing membrane, was placed over the furcation defect. Mucoperiosteal flaps were sutured back using interdental interrupted sutures. Surgical procedure for control group was identical to the test group (β-TCP + except the omission of placement of PRF. Antibiotic coverage (Amoxicillin 500 mg three times a day) and analgesic (Ibuprofen 400 mg and Paracetamol 325 mg three times a day) was prescribed for 5 days post-surgical period. Patients were instructed to rinse twice daily with 0.12% chlorhexidine gluconate for 6 weeks. A complete post-operative evaluation was performed at 12 month follow up visit and all the clinical parameters were re-assessed.

**Statistical analysis**

Student’s paired t-test was used to compare data from baseline to those at 12 months for each treatment group. A comparison between treatment groups at baseline and 12 months post-surgery was accomplished with student’s unpaired t-test.

**Results**

Out of 24 class II furcation defects, 22 defects were located on buccal surfaces while 2 were located on lingual surfaces of mandibular molars. There were no untoward effects, allergy, infection or patient complaints related to graft material. None of the selected patients dropped out before the termination of the study.

In general patients showed good oral hygiene throughout the study. Baseline full mouth mean plaque index (PI) score was 0.82 ± 0.13, which at 12 months decreased to 0.67 ± 0.13 in the test group. Baseline full mouth mean plaque index (PI) score was 0.80 ± 0.11, which at 12 months decreased to 0.65 ± 0.12 in the control group. The difference from baseline to 12 months in the test group (0.15 ± 0.001) as well as the control group (0.15 ± 0.001) was found to be statistically significant post-surgical measurement was statistically significant.

The mean papilla bleeding index (PBI) score during 12-month period remained low (<1). Baseline full mouth mean papillary bleeding index (PBI) score was 0.73 ± 0.10, which at 12 months decreased to 0.60 ± 0.09 in the test group. Baseline full mouth mean plaque index (PI) score was 0.71 ± 0.14, which at 12 months decreased to 0.54 ± 0.11 in the control group. The difference from baseline to 12 months in the test group (0.13 ± 0.08) as well as the control group (0.17 ± 0.10) was found to be statistically significant post-surgical measurement was statistically significant.

**Baseline Characteristics of the sites in the treated groups**

The baseline defect characteristics are presented in Table 1. At baseline, no statistically significant differences in any of the investigated parameters were observed between the test and control groups, indicating that the randomization process was effective.

**Clinical Outcomes at 12 Months**

Student’s paired t-test indicated that both the test (HA and β-TCP+PRF) and control (HA and β-TCP) groups showed significantly greater mean PPD reduction of 2.0 ± 0.73 mm and 0.50 ± 0.52 mm respectively at 12 months compared to baseline (Table 1).

On analysis of differences in mean PPD reductions for the test group versus control group at 12 months by Student’s unpaired t-test, statistically significant greater probing depth reduction of 1.50 ± 0.90 mm was observed in the test group as compared to the control group.

The observed differences between baseline CAL and 12 months...
CAL were analyzed by Student’s paired t-test, and were found to be statistically significant in both groups. When the differences in CAL gains for the test group (3.0 ± 0.95 mm) versus control group (2.00 ± 0.85 mm) were analyzed by Student’s unpaired t-test, statistically significant difference was observed in favor of the test group (Table 1). The mean CAL gain observed in the test group was 1 ± 1.20 mm greater than the control group.

Increase in gingival recession was not found to be statistically significant in the test (1.0 ± 1.12 mm) as well as the control group (1.34 ± 1.07 mm), when compared to baseline (Table 1). However, no statistically significant difference was found in increase in gingival recession (0.34 ± 1.43 mm) between the test and control groups.

Statistically significant reductions of horizontal probing depth were recorded for the test group as well as for the control group (Table 1). When comparison was made between the test and control groups using the Student’s unpaired t-test, statistically significant greater reduction of horizontal probing depth was observed in test group, with additional benefit of 0.34 mm (Table 1).

The Frequency analysis of furcation changes

Complete furcation closure was achieved at 6 sites (50%) in HA and β-TCP + PRF group as compared to 2 sites (16.66%) in the HA and β-TCP group. The improvement in horizontal classification from class II to class I at 6 months post-operatively. The mean PPD reduction obtained in test group was 2.0 mm while as in control group it was 0.50 mm. A statistically significant greater reduction of mean PPD (1.50 mm) was observed in test group compared to the control group. The mean PPD reduction observed in the present study by using β-TCP + PRF are comparable with other studies reported in the literature on use of PRF in combination with bone grafts. Panda et al.15) reported in their case report mean PPD reduction of 6 mm following application of PRF in combination with an alloplast (Ossifi) for the treatment of infrabony defects at 6 months post-surgery. Sambhav et al.16) reported mean PPD reduction of 4 mm following use of PRF in combination with β-TCP for the treatment of Grade II furcation defect at 6 months. Sharma et al.17) reported mean PPD reduction of 4.06 mm after 9 months following application of Autologous PRF alone in the treatment of class II furcation defects. The greater mean probing pocket depth reduction reported by pervious investigators could be explained by the greater initial probing pocket depth in their studies.

Primary efficacy parameters for validation of clinical periodontal regeneration after periodontal therapy is gain in clinical attachment level (mm)18,21). The outcome measures to assess periodontal regeneration of furcation treated sites, it has become increasingly common to measure attachment gain in vertical and horizontal direction, since the primary goal of furcation therapy is to reduce the magnitude of furcation defect to a size, which is maintainable by routine hygiene method and mechanical instrumentation18,21). In the present study, β-TCP + PRF group showed statistically significant clinical attachment gain of 3 mm compared to β-TCP group (2 mm) at 6 months. Observations made in the present study with regards to clinical attachment level gain are comparable with results reported in the previous studies. Sharma et al.22) evaluated the effectiveness of autologous PRF in the treatment of grade II furcation defects and reported gain in clinical attachment level by 2.33 mm at 9 months. Thorat et al.23) in their single center controlled clinical trial investigated the effectiveness of autologous PRF in the treatment of infrabony defect and reported gain in clinical attachment level of 3.69 mm at 9 months.
In the present study, significant reduction in mean horizontal probing depth was observed in β-TCP + PRF group (3.33 mm) as well as in β-TCP alone group (1.75 mm). When comparison was made between two groups, significantly greater HPD Reduction was observed in the β-TCP + PRF group (1.58 mm).

The main clinical end point of any given periodontal therapy for the treatment of furcation lesion is the full closure of furcation, or if this cannot be attained then the conversion of deep into shallow lesion. However, the predictability of these treatment goals is influenced by several factors like patient compliance, defect selection, treatment techniques and others. Thus, there is a need to better understand factors and conditions which may have an influence upon treatment outcome. Consequently, in the present study, analysis was performed in relation to treatment modality. In the present study, the number of class II furcation defects that closed or converted to class I were higher in β-TCP + PRF group compared to β-TCP alone group. At present, there are no data available in the literature on the use of PRF in combination with β-TCP for the treatment of class II furcation defects indicating frequency of clinical furcation changes.

Although there is a sparsity of data on gingival recession following regenerative therapy with the use of β-TCP + PRF for the treatment of furcation defect, the present study showed gingival recession of 1 mm in β-TCP + PRF group and 1.34 mm in β-TCP alone group. When GR was compared in both groups the difference was non-significant (0.34 mm). Findings in the present study with regards to post-surgical gingival recession were compared with previously reported studies. Thorat et al. in a single center controlled clinical trial investigated the effectiveness of autologous PRF in the treatment of infrabony defects and reported 0.81 mm marginal tissue recession after surgery. Sharma and Pradeep evaluated effectiveness of autologous PRF in the treatment of infrabony defects and reported 0.79 mm marginal tissue recession at 9 months post-surgery.

The most reliable outcome variable for assessing periodontal regeneration is human histology. Due to ethical consideration and patient management limitation, no histologic evidence was obtained to establish the proof of true periodontal regeneration. The application of PRF in management limitation, no histologic evidence was obtained to establish the quality of the regenerated tissue.

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Conflict of Interest

The authors have declared that no COI exists.

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