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) alone 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. 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 results3,4.

It was suggested that the combination treatment including both bone replacement graft + GTR would provide the most beneficial regenerative therapy for class II furcation defects5. 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 membrane6. However, the results obtained in controlled clinical studies demonstrated that the use of bone graft together with barrier membrane was of limited significance1,8. 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 fill9. 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.7 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 in multiple gingival recessions with coronally displaced flap and in facial plastic surgical procedures. Beta tricalcium phosphate (β-TCP) is a purified, multicrystal, porous form of calcium phosphate with Ca:PO₄ 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.

Recently Lekovic et al. 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 (Ossifi, 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 epiinphrine) 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.

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 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
Table 1. Comparison of measured variables for test and control group

<table>
<thead>
<tr>
<th>Examination interval</th>
<th>PPD (Mean ± SD in mm)</th>
<th>CAL (Mean ± SD in mm)</th>
<th>HPD (Mean ± SD in mm)</th>
<th>REC (Mean ± SD in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>Baseline</td>
<td>3.16 ± 0.57</td>
<td>2.66 ± 0.49</td>
<td>11.41 ± 0.66</td>
<td>10.41 ± 0.66</td>
</tr>
<tr>
<td>At 12 months</td>
<td>1.16 ± 0.38</td>
<td>2.16 ± 0.57</td>
<td>8.41 ± 0.56</td>
<td>8.41 ± 0.56</td>
</tr>
<tr>
<td>Difference</td>
<td>2.00 ± 0.73</td>
<td>0.50 ± 0.52</td>
<td>3.33 ± 0.83</td>
<td>2.00 ± 0.85</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001 S</td>
<td>0.006 S</td>
<td>0.001 S</td>
<td>0.001 S</td>
</tr>
</tbody>
</table>

Table 2. Frequency of clinical furcation changes at 6 months in β-TCP and β-TCP + PRF group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group (β-TCP Group)</th>
<th>Test Group (β-TCP + PRF Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete closure (HPD = 0)</td>
<td>2 (16.66%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Changes from Class II to Class I (HPD&lt; 3)</td>
<td>6 (50%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>No change</td>
<td>4 (33.34%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

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 was observed at 6 sites (50%) in the HA and β− TCP + PRF group and 6 sites (50%) in HA and β− TCP group. However, 4 sites (33.34%) in HA and β− TCP group remained unchanged (Table 2).

Discussion

Successful regeneration of periodontal furcation defects is clinically defined as the complete elimination of horizontal and vertical defect components by bone fill and improvement in the clinical parameters. For the treatment of mandibular class II furcation defects, extensive studies using diverse materials and therapies have been performed. While some reports demonstrated significant clinical attachment and bone gains, other studies did not show clinical improvement. Thus, the present investigation was undertaken to compare the effectiveness between PRF in combination with β−TCP with that of β−TCP alone for the treatment of mandibular class II furcation defects in human.

The present study was conducted over a period of 6 months using randomized parallel design. The results in the present studies were obtained by comparing test group (β−TCP + PRF) with a control group (β−TCP alone). At baseline, none of the investigated parameters at the sites treated by β−TCP and β−TCP + PRF showed any statistical difference, thus ensuring the same starting point for both the procedures tested. There was no sign of allergy, infection or any other complication in any patients, after the use of β−TCP as well as PRF. During 6 months observation period, the wound healing was uneventful. None of the selected patients dropped out before the termination of the study and all the patients were satisfied with the treatment modalities provided to them.

The results of present clinical and radiographic study support the concept that the use of PRF in combination with β−TCP provided added benefits of clinical and statistical significance to the treatment of furcation defects. In fact, the clinical attachment gains, the probing pocket depth reduction and horizontal defect depth reduction, were consistently greater than those observed in sites treated with β−TCP alone. Reduction of probing pocket depth in order to limit the risk of local reinfection is a primary goal of periodontal therapy. Shallow pockets have a strong negative predictive value for future disease progression, while deep pockets in treated areas are risk indicators for periodontal disease progression. In the present study, both the groups showed reduction of probing pocket depth at 6 months post-operatively. The mean PPD reduction obtained in test group was 2.00 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 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. reported in their case report mean PPD reduction of 6 mm following application of PRF in combination with an alloplast (Ossifit) for the treatment of infrabony defects at 6 months post-surgery. Sambhav et al. 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. 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. 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 instrumentation. 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. 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. 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.
The authors have declared that no COI exists.

Acknowledgement

We would like to thank specialist biostatistician for statistical analysis help.

Conflict of Interest

The authors have declared that no COI exists.

References


