Treatment of Mandibular Molar Class II Furcation Defects in Humans With Bovine Porous Bone Mineral in Combination With Plasma Rich in Growth Factors

S. Sadat Mansouri¹, M. Ghasemi¹, S. Saljughi Darmian², T. Pourseyediyan²

¹Associate Professor, Department of Periodontics, Faculty of Dentistry, Islamic Azad University, Tehran, Iran
²Dentist, Tehran, Iran

Abstract

Objective: The purpose of the present randomized clinical trial study was to compare the effectiveness of Bovine Porous Bone Mineral (BPBM) with and without Plasma Rich in Growth Factors (PRGF) in the treatment of mandibular Class II furcation defects.

Materials and Methods: In seven patients, nine pairs of symmetric buccal or lingual mandibular class II furcation defects were treated. In each patient, one defect received BPBM (control) and the other received BPBM/PRGF (test) by random assignment. Clinical measurements were made both at baseline and 6-month evaluation.

Results: Similar improvements were observed with both treatment modalities. Significant reductions were gained in the gingival index, probing depth and relative vertical clinical attachment level. Plaque index and gingival recession changes were not significant in both groups. The mean probing depth reductions were 2.67±0.87 mm for the control group and 3.22±1.56 mm for the test group (p<0.001). The mean relative vertical clinical attachment level gains were 1.57±0.96 mm (p<0.001) and 1.65±1.24 mm (p<0.004) in the control and test groups, respectively. In the test group, the relative horizontal clinical attachment level reduced from 5.87±0.96 mm to 4.58±1.02 mm (p<0.02). No significant differences were observed in all clinical parameters 6 months postoperatively between the two groups.

Conclusion: The application of a combined technique using BPBM/PRGF, compared to the BPBM alone, resulted in greater healing, although not significant, in the treatment of mandibular class II furcation defects.

Key Words: Furcation Defect; Platelet-Rich Plasma; Bone Transplantation; Regeneration

INTRODUCTION

Furcation involvements present difficult treatment problems due to the complex morphology of the area, which is considered as one of the challenging aspects of periodontal therapy [1-2]. The final goal associated with periodontal therapies was the supporting apparatus replacement through the process of cementum, periodontal ligament and alveolar bone regeneration [3-4]. The combination of a graft
material and Guided Tissue Regeneration (GTR) has been shown most desirable outcome of periodontal regeneration in furcations [5-8]. It has been shown that, while clinical attachment levels may be improved with GTR alone, bone grafts play a positive role in promoting defect fill with hard tissue, a favorable event in periodontal regeneration. Bovine Porous Bone Mineral (BPBM) is one of the graft materials. It is prepared by bone extraction of bovine bone, that results in a trabecular structure similar to human cancellous bone and can enhance bone formation [9]. Polypeptide growth factors (PGFs) are biologic mediators that regulate human cell proliferation, chemotaxis and differentiation. There has been an increased interest in the applications of PGFs to periodontal regenerative procedures [10]. Of all PGFs known, platelet-derived growth factor (PDGF) has been shown to exert a favorable effect on periodontal regeneration [11]. PDGF and Transforming Growth Factor β (TGFβ) are abundant in the alpha granules of platelets [12]. The application of PRP to the wound-healing site increases the concentration of platelets by up to 338% [13]. The use of PRP, BPBM and GTR combined technique was an effective modality of regenerative treatment for intrabony defects and furcation defects [12,14-18]. PRGF such as PRP is rich in growth factors and presented as a tissue regeneration technique [19]. Due to the lack of comparative studies on the use of PRGF in combination with graft materials as BPBM, the purpose of this study was to investigate the effects of BPBM/PRGF in the treatment of mandibular Class II furcation defects.

MATERIALS AND METHODS

Study Population

Seven patients (4 males and 3 females, the mean age of 44.7±11.2 years), undergoing periodontal therapy at the Department of periodontology in Tehran Azad University took part in the present study. Each patient exhibited one pair of similar contralateral Class II furcation defects. All subjects were advised of the purpose of the study and signed an informed consent form approved by the committee for the protection of Human Subjects at the Tehran Azad University. All patients were enrolled from March 2008 to May 2009. The inclusion criteria for the study included the presence of paired, contralateral mandibular Class II furcation defects with a probing depth ≥ 5 mm and a horizontal probe penetration ≥ 3 mm [20]. Patients with any systemic illness, compromised immune system, pregnancy and lactation, smoking, those taking drugs known to cause gingival enlargement, allergy or sensitivity to any medication to be used in the study were not included. Endodontically treated teeth were also excluded from the study.

Study Design

This study was a split-mouth randomized clinical trial. Presurgical treatments included oral hygiene instruction, scaling and root planing were administered for the patients. Plaque control was repeated until O’Leary Plaque Index ≤ 25% was obtained [21]. Occlusal adjustment was performed if trauma from occlusion was diagnosed. Trauma from occlusion was evaluated by examining the obvious presence of fremitus in centric occlusion or in working or balancing excursions. 6-8 weeks following phase I therapy, a periodontal re-evaluation was performed to confirm the suitability of the sites for the study. The selected sites were divided randomly (toss of coin) into control and test groups.

Clinical Measurements

The following parameters were measured in both groups on the day of the surgical procedure and 6 months later using the same type of probe.‡ Both Plaque Index (PI) [22] and Gingival Index (GI) [23] were recorded. Probing Depth (PD) using the gingival margin as refer-
ence was measured. Gingival Recession (GR), present in the furcation area was measured from the cementoenamel junction (CEJ) to the gingival margin. Relative Vertical Clinical Attachment Level (RVCAL), using the acrylic stent as reference, was measured with customized acrylic stents using grooves to ensure a reproducible placement of the periodontal probe.

In order to ensure the correct and repetitive insertion of the stent, the distance between CEJ and stent edge was also recorded. To measure Relative Horizontal Clinical Attachment Level (RHCAL), the periodontal probe was inserted into the furcation area, perpendicular to a horizontal one which was placed on the buccal or lingual aspect of the involved tooth and measured using a digital caliper; §

**PRGF Preparation**

At the time of the surgery, 9 ml of blood was drawn from each patient using 5 ml tubes, which contained 3.8% sodium citrate as anticoagulant.

The blood containing tubes were shaken gently to enhance complete mixing of the blood with the anticoagulant.

Then, the glass tubes containing the blood were centrifuged by a digital machine ‖ at 460 G for 8 minutes. As a result, plasma was separated into different fractions: At the top, 1cc of PPGF (Plasma Poor in Growth Factors), in the middle, 0.5 cc of PGF (Plasma average in Growth Factors) and at the bottom, PRGF 0.5 cc of plasma rich in growth factors, exactly over red blood cells. We carried out a more careful pipetting, this time using 100µl (0.1cc) pipette, to prevent the occurrence of any turbulence and avoid the aspiration of red blood cells. We performed the pipetting 5 times (0.1cc×5=0.5cc) and transfer the PRGF to sterile tube.

**Surgical Procedure**

One surgeon performed all surgeries. Following the administration of local anesthesia (Lidocaine 2 % with Epinephrine 1:80, 000), an intrasulcular incision was made and mucoperiosteal flaps were raised. Care was taken to preserve as much inter-proximal soft tissue as possible. Meticulous defect debridement and root planing were carried out using curettes ¶ and ultrasonic instruments. # No osseous recontouring was done.

A full thickness flap was also raised opposite the involved furcation, using the same technique, in order to rule out a Class III furcation defect.

The diagnosis of a Class III furcation invasion at the time of the surgery, disqualified that tooth from the study.

Treatment of the furcation defects in the test group was as follows: Plasma was activated using 10 % Calcium Chloride. 50 µl of 10 % calcium chloride was added to sterile tube containing 1 ml of PRGF. Following 5 to 7 minutes at room temperature or 2 to 3 minutes at 37 °C (in Heatblock), a PRGF gel was formed. Bovine Porous Bone Mineral granules, ** particle size 0.25-1.0 mm, were mixed with the coagulated PRGF preparation at a proportion of 1:1. Prior to grafting, PRGF and the coagulating solution were applied to the defect walls and root surfaces.

The BPBM/coagulated PRGF mixture was then tightly packed in the furcation area using amalgam condensers vertically to the roof of the furcation [12]. Flaps were sutured at the original levels using 4-0 non-absorbable black braided silk surgical suture. †† Interrupted sutures were placed. Surgical procedure in the control group was the same as that of the test group except for the use of BPBM, alone. Both surgical sites were covered with periodontal dressing. ‡‡
Postoperative Care
Antibiotic (Amoxicillin 3× 500/day) was prescribed for 7 days. Analgesics such as Ibuprofen 800 mg, three times per day, were also prescribed, if necessary. Patients were asked to rinse their mouth with 0.20 % chlorhexidine gluconate mouthrinse twice daily for the first 2 weeks following the surgery, and they were instructed not to brush or floss in the areas where surgery had been performed for 2 weeks.

Periodontal dressing and sutures were removed one week postoperatively. Patients were examined weekly up to 1 month after surgery and then at 3 and 6 months. Prophylaxis was performed during this period if necessary.

At 6 months postoperatively, all clinical measurements were re-evaluated.

Statistical Analysis
All clinical parameters were tested for normal distribution using the Kolmogorov-Smirnov test. For the comparison of test and control treatments, the changes from baseline to 6-months later were calculated. For the statistical evaluation of the changes from baseline to 6-months in each treatment group, the paired t-test was used. For comparisons between the groups, the un-paired t-test was used. Due to the high variability of results in GI and PI, these differences for test and control groups were compared using the non-parametric Wilcoxon Signed Ranks Test. Statistical significance was set at 0.05. Statistical analysis was performed using a commercially available software program.

RESULTS
All patients completed treatment and post-treatment phases successfully. Healing was performed satisfactorily. No allergic reactions, abscess, infection and necrosis were seen. Changes in probing depth, gingival recession, and relative vertical and horizontal clinical attachment level are shown in Table 1 and 2.

The mean presurgical PD was similar for control and test sites. At 6 months postoperatively, the mean PD reduction in the BPBM group was 2.67±0.87 mm (50% reduction) and in the BPBM/PRGF group was 3.22 ± 1.56 mm (57 % reduction) (P<0.001).

Table 1. PD, GR at Baseline and 6 Months (mean ±SD [mm])

| Group          | PD*          | GR†          |
|----------------|--------------|--------------|
|                | Base line    | 6 months     | Difference | P Value | Base line    | 6 months     | Difference | P Value |
| BPBM           | 5.33±0.71    | 2.67±0.71    | 2.67±0.87   | <0.001‡  | 1.33±0.5     | 1.67±0.87    | 0.33±0.87   | <0.28   |
| BPBM/PRGF      | 5.67±1.32    | 2.44±0.73    | 3.22±1.56   | <0.001‡  | 1.56±1.51    | 2±1          | 0.44±0.73   | <0.10   |
| P Value        | <0.51        | <0.60        | <0.37       | <0.68    | <0.46        | <0.77        |

(n=9 for each treatment group)
Bovine Porous Bone Mineral (Control)
Bovine Porous Bone Mineral plus Plasma Rich in Growth Factors (Test)
*Probing Depth
†Gingival Recession
Statistically significant difference (P <0.05)‡
not significant (P >0.05)
After 6 months, PD reductions were similar between the groups (P<0.37). The mean GR, at baseline and 6 months postoperatively, was similar for control and test sites. The mean RVCAL was the same at baseline and 6 months following surgery (P<0.88). The CAL gains, in the BPBM and BPBM/PRGF groups were 1.57±0.96 mm and 1.65±1.24 mm, respectively which were statistically significant. The mean presurgical RHCAL was similar for control and test sites. At 6 months postoperatively, the mean changes of RHCAL in BPBM and BPBM/PRGF groups were 0.66±1.42 mm (12% reduction, P<0.20) and 1.29±1.30 mm (2% reduction, P<0.02), respectively. After 6 months the changes were similar between the groups (P<0.34).

Plaque index and gingival index changes are shown in Table 3. PI, at baseline and 6 months postoperatively and also its changes were similar in both groups. GI, at baseline and 6 months postoperatively and its changes were similar between groups and showed a significant reduction in both groups.

The complete closure of furcation defects was not seen in any of the groups.

**DISCUSSION**

The results of the present study have shown that BPBM application with or without PRGF was found to be effective in treating furcation defects leading to a significant reduction in GI, PD and CAL gain. Moreover, probe horizontal insertion, in furcation site, reduced significantly in the BPBM/PRGF group, although the difference between the groups was not significant. Lekovic et al [12] through the use of (PRP/BPBM/GTR) as compared with Open Flap Debridement (OFD), in treating mandibular Class II furcation defects, showed that the probing depth reduced 4.07±0.33 mm in the combination group and 2.49±0.38 mm in the OFD group. CAL gains were 3.29±0.42 mm and 1.68±0.31 mm in the two groups, respectively. The combined therapy that was used by Lekovic et al does not allow any conclusion to be made regarding the component(s) which was responsible for the improvement observed in the triple combination therapy. Although the number of samples in this study was less than that of Lekovic et al, it was observed that the use of graft materials with or without PRGF could cause 3.22±1.56 mm and 2.67±0.87 mm PD reductions, respectively and 1.6 mm CAL.

**Table 2. RVCAL, RHCAL at Baseline and 6 Months (mean ±SD [mm])**

| Group              | Base line  | 6 months | Difference  | P Value | Base line  | 6 months | Difference | P Value |
|--------------------|------------|----------|-------------|---------|------------|----------|------------|---------|
| BPBM               | 9.87±1.41  | 8.31±1.11| 1.57±0.96   | <0.001‡ | 5.51±1.28  | 4.85±1.21| 0.66±1.42  | <0.20   |
| BPBM/PRGF          | 10.30±2.01 | 8.65±1.44| 1.65±1.24   | <0.004‡ | 5.87±0.96  | 4.58±1.02| 1.29±1.30  | <0.02‡  |
| P Value            | <0.61      | <0.60    | <0.88       | <0.51   | <0.80      | <0.34    |            |         |

(n=9 for each treatment group)

Bovine Porous Bone Mineral (Control)
Bovine Porous Bone Mineral plus Plasma Rich in Growth Factors (Test)
Relative Vertical Clinical Attachment Level*
Relative Horizontal Clinical Attachment Level†
‡Statistically significant difference (P <0.05)
not significant (P >0.05)
gains in both groups. Even though BPBM/PRGF group showed more PD reduction, the difference between the two groups was not significant. Less PD reduction and CAL gain in this study as compared to Lekovic et al’s study, may be attributed to GTR application in the PRP/BPBM/GTR group in Lekovic et al’s study. In a research conducted by Reddy et al [24] in two groups of an anorganic bovine xenograft plus 10% collagen (BO) and (BO) combined with bioresorbable collagen barrier (BO/BG), PD reductions were 2.1±0.74 mm and 2.6±0.7 mm in the groups, respectively; whereas, the amounts of CAL gain were 1.8±0.63 mm in BO group and 2.5±0.71 mm in BO/BG group. The equal CAL gain amounts of BO group in Reddy et al’s study and those of the present study, indicated the effective role played by graft materials (BPBM) in PD reduction and CAL gain. Tsao et al [25] compared mineralized human cancellous bone allograft (MBA) with MBA/GTR and OFD and showed that there was a significant 0.9 mm reduction in PD in the MBA group. PD reduction in MBA/GTR was only 0.7 mm and not significant. CAL gain in the MBA group which showed the best results in PD reduction, was 0.1 mm that was much less than that of the present study attributing a more desirable effect to BPBM in comparison with MBA. Comparing the results of the present study with that of Eickholz et al’s study [26] which investigated the long-term effects of GTR by absorbable and non-absorbable membrane in treating class II furcation defects, PD reduction and CAL gain, in a 12-month study of both groups, were less than the present study indicating that single application of graft materials would be more promising than single use of GTR. Another research was carried out by Meyle et al [27] on the effects of Enamel Matrix Derivative or membrane in treating class II furcation defects. Although the regenerative consequences were somehow similar between the two groups, PD reduction and CAL gain were less, as compared with the present study. Therefore, BPBM application with or without PRGF may exert more desirable effects on furcation defects.

Regarding intrabony defects, a combined technique using PRP with bone graft has been proved to be more effective on PD reduction and CAL gain, as compared with bone graft alone [16, 18].

In the present study, although PD reduction and CAL gain were proved to be higher in the test group indicating the positive effects of

Table 3. GI, PI at Baseline and 6 Months

| Group                    | GI*  | PI†   |
|-------------------------|------|------|
|                         | Baseline | 6 months | Difference | P Value | Baseline | 6 months | Difference | P Value |
| BPBM                    | 1.61±0.22 | 0.50±0.66 | 1.11±0.70 | <0.001† | 0.78±0.32 | 0.67±0.41 | 0.11±0.25 | <0.23 |
| BPBM/PRGF               | 1.61±0.22 | 0.28±0.26 | 1.33±0.25 | <0.0001‡ | 0.72±0.34 | 0.69±0.41 | 0.03±0.19 | <0.68 |
| P Value                 | <1.00  | <0.40  | <0.38    | <0.72   | <1.00    | <0.45    |

(n= 9 for each treatment group)

Bovine Porous Bone Mineral (Control)
Bovine Porous Bone Mineral plus Plasma Rich in Growth Factors (Test)
*Gingival index of Löe & Silness (1963)
†Plaque index of Silness & Löe (1964)
Statistically significant difference (P <0.05) ‡
not significant (P >0.05)
PRGF and growth factors, the significant difference was not observed between the two groups, which can be attributed to the low number of samples in the present study and the properties of the furcation site in comparison with the intrabony defects. Reduction in pocket depth may be the most important outcome of any periodontal procedure, including periodontal regeneration, because it directly affects the ability of a clinician to maintain a treated site [25]. The final mean PD for both groups in this study is considered maintainable in clinical practice. CAL gain might have been a result of true periodontal regeneration via new attachment or alternatively, of healing by repair, which implies the presence of a long junctional epithelium between the newly regenerated tissues and the root surface [12]. Therefore, histological studies are the only reliable method to determine the nature of the periodontal soft and hard tissue interface. PD and CAL are parameters evaluated by inserting probe into a sulcus or pocket [12]. So it should be noted that graft placement may influence tissue consistency and inhibit probe insertion without the presence of any CAL gain promotion [12]. PD reduction values in BPBM/PRGF and BPBM groups were 57% and 50%, respectively which were significant comparing to that of baseline and CAL reduced 16% in both groups of the present study which were statistically significant. Defect fill values, due to the lack of reentry surgery were evaluated through probe horizontal insertion into furcation prior and after surgery showing RHCAL gain as 22% for BPBM/PRGF group that was significant and 12% for BPBM group that was not significant. Although no significant difference was found between two groups after 6 months, higher RHCAL gain in BPBM/PRGF group up to 10% could be the result of PRGF positive properties such as sticky consistency that due to its abundant fibrin content exhibits ideal consistency following mixture with BPBM and improved clinical healing. Fibrin may enhance haemostatic activity and blood-clot stability [12, 28]. Moreover, it can also inhibit apical movement of epithelial and connective tissue [29]. Mean GR changes, in the present study were shown to be 0.44±0.73 mm and 0.33±0.87 mm in BPBM/PRGF and BPBM groups, respectively. However, the difference was in accordance with other studies [12,24,25] and was not statistically significant. In the research by Meyle et al [27], GR, at membrane site was found to be a little more than that of EMD. Therefore, due to the lack of GR in groups of the present study, it could be concluded that PD reduction was not the result of apical migration of the margin and CAL gain is expected to have an influence on this reduction. In the present study, the type of tooth (first or second molar) and furcation site (buccal or lingual) did not influence CAL gain and PD reduction.

CONCLUSION
Within the limits of the present study, despite the statistically nonsignificant results, it seems that PRGF use in combination with the graft materials are beneficial in furcation defect treatment leading to a higher PD reduction and CAL gain as compared with graft materials alone. Studies with larger sample sizes and histological evaluations are recommended.

‡ 15-mm University of North Carolina (UNC-15) periodontal probe, Hu-Friedy, Chicago, IL.
§ Absolute Digimatic, Mitutoyo Corp., Japan.
‖ PRGF system-Biotechnology institution-Spain.
¶ Gracey, Hu-Friedy, Chicago, IL, USA.
# Cavitron, Dentsply, Tulsa, OK, USA.
** Bio-Oss®, Geistlich, Wolhusen, Switzerland
†† Ethicon, Johnson and Johnson Ltd., Somerville, NJ, USA
‡‡Coe-Pak, GC America Inc., Chicago, IL, USA.
§§ SPSS® version 16, SPSS Inc., Chicago, IL, USA.
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