CLINICAL AND CONE-BEAM COMPUTED TOMOGRAPHY EVALUATION OF XENOGRAFT ALONE OR IN COMBINATION WITH PLATELET RICH FIBRIN IN THE TREATMENT OF GRADE II MANDIBULAR FURCATION INVOLVEMENT

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ABSTRACT

Aim of study: The aim of this study was to compare the effect of Xenograft alone or in combination with Platelet rich fibrin (PRF) in the treatment of mandibular furcation grade II defects.

Patients and methods: Twenty patients with a total number of 40 grade II furcation defects in split mouth design determined clinically and radiographically were selected for the study. The selected sites were treated with open flap surgery and divided into two groups: Group I was treated with Xenograft (cerabone®) only and Group II treated with Xenograft (cerabone®) plus PRF membrane. The clinical and radiographic evaluation occurred at baseline (before surgery) and 6 month post-operative. Clinical parameters include: plaque index (PI), gingival index (GI), vertical clinical attachment level (VCAL) and Horizontal clinical attachment level (HCAL). Cone-beam Computed Tomography (CBCT) was performed to measure furcation height (FH), width (FW), and depth (FD) defects of mandibular molars at baseline (before surgery) and 6 month post-operative.

Results: Both groups showed statistically significant reduction in all clinical and radiographic parameters within groups from baseline to 6 months post-operative. HCAL, FH, FW and FD were significantly increased in G II compared to G I at 6 months post-operative.

Conclusion: Treatment of furcation grade II defects with xenograft (cerabone®) plus PRF led to significant improvement more than xenograft (cerabone®) alone.

KEYWORDS: CBCT, platelet-rich fibrin (PRF), xenograft, furcation grade II defect.

INTRODUCTION

Periodontitis is a chronic multifactorial inflammatory disease associated with plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus. Its primary features include the loss of periodontal tissue support, manifested through clinical attachment loss (CAL) and radiographically assessed alveolar bone loss, presence of periodontal pocketing and gingival bleed-

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ing. Periodontitis is a major public health problem due to its high prevalence, as well as because it may lead to tooth loss [1].

Definite periodontal pathogens and associated host-mediated immune responses cause progressive loss of the tooth-supporting tissues which ultimately result in the formation of intraosseous defects or furcation defects. Furcation defect refers to the invasion of the bifurcation and trifurcation of multirooted teeth by periodontal disease, which offers a unique challenge for its treatment both from a prognostic perspective and from the perspective of therapeutic measures. The ideal goal of furcation therapy is to completely close the furcation defect and retain the tooth, there by achieving the local condition to one of anatomic normalcy, facilitating long-term periodontal maintenance therapy, and the likelihood of tooth retention [2].

Grade II furcation defects has traditionally been treated by closed scaling and root planning, in addition to resective osseous techniques, but the results are largely unpredictable [3]. Regeneration of furcation defects has been reported following a variety of surgical approaches involving root surface conditioning. The placement of bone grafts or bone substitute implants, or the use of organic or synthetic barrier membranes (guided tissue regeneration), biological mediators and growth factors are considered successful [4].

Bone regeneration can be accomplished through three different mechanisms: osteogenesis, osteoinduction, and osteoconduction. The primary types of bone graft material are autogenous bone, allografts, xenografts and alloplasts. Autogenous bone harvested from the patient forms new bone by osteogenesis, osteoinduction and osteoconduction. Allografts harvested from cadavers have osteoconductive and possibly osteoinductive properties, but they are not osteogenic. Xenografts/alloplasts are typically only osteoconductive [5].

Xenograft (cerabone®) is bovine porous bone mineral that recently used in periodontal regenerative procedures. This material is prepared by protein extraction of bovine bone, which results in a structure similar to human cancellous bone and has ability to enhance bone formation. Because of the widely reticulated interconnecting pores and the small crystals, the internal surface of this material covers the area similar to that of human spongy bone. This has enabled an extremely close contact with newly formed bone. Bone formation has been shown with bovine porous bone mineral in a variety of periodontal applications, including ridge augmentation, repair of vertical defects, sinus elevations, and guided bone regeneration around implants [6].

cerabone® is natural bovine bone grafting material which is derived from bovine bone in an established high-temperature heating process (sintering) ensuring high safety. Beside safety and reliability of the product and the production process, the material fulfills all other important requirements for the clinical success of a bovine bone graft material. These characteristics are the basis for the excellent clinical results of cerabone® demonstrated by high volume stability of the grafted site, complete integration into newly formed bone matrix and the resulting high bone density [7].

Some biological mediators such as enamel matrix derivatives, platelet-rich plasma (PRP), platelet-rich fibrin (PRF), platelet-derived growth factor, and bone morphogenetic proteins has opened new possibility in the treatment of furcation defects. Choukroun’s PRF, a second-generation platelet concentrate, is an intimate congregation of cytokines, glycemic chains, and structural glycoproteins trapped within a fibrin network with synergetic effects on healing processes [8, 9]. Favorable effects of PRF have been studied in various procedures, such

* Cerabone®: botiss biomaterials, GmbH, 15806 zossen, Germany.
as facial plastic surgery\cite{10}, sinus-lift procedure\cite{11}, multiple gingival recession (GR) with a coronally displaced flap\cite{12} and furcation defects and intrabony defect\cite{13,14}.

According to Chang et al. PRF can promote the healing of osseous defects by promoting the expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and stimulates the production of osteoprotegerin (OPG) which in turn causes proliferation of osteoblasts\cite{15}.

Most of the bone grafts are osteoconductive in nature. Thus, the adjunct use of PRF may be useful in bone regeneration due to its osteoinductive properties\cite{16}.

So in this study, it’s proposed to assess adjunctive use of PRF with cerabone® bone grafting material in comparison of using cerabone® material only in the treatment of furcation grade II defects.

PATIENTS AND METHODS

1: Selection of patients

The current study included twenty systemically healthy subjects undergoing periodontal therapy at the Out Patients Clinic, Department of Oral Medicine, Oral Diagnosis and Periodontology, Faculty of Dentistry, Minia University.

The study inclusion criteria were:

The presence of furcation grade II defects classified according to Ramfjord and Ash 1979 \cite{17} in vital, asymptomatic molar teeth with a radiolucency in the furcation area, vertical probing depth (VPD) ≥5mm and horizontal clinical attachment level (HCAL) ≥3mm following phase I therapy (scaling and root planning). Patient selection was performed according to the Cornell medical index \cite{18}. Patients were re-evaluated after phase-I therapy, teeth with inter-proximal intra-bony defects, gingival recession, endodontic involvement or mobility Grade II of Miller’s classification \cite{19} were also excluded.

II) Ethical regulations:

The complete treatment plan was explained to all patients including detailed steps, risks, and expected results and their full signed consent was obtained prior to entry into the study. The study was complied with the rules set by the International Conference on Harmonization Good Clinical Practice Guidelines, and the Declaration of Helsinki and the research ethics committee of the Faculty of Dentistry, Mini University \cite{42}.

III) Treatment protocol:

All patients underwent phase I therapy comprising of full mouth mechanical debridement including supra and sub-gingival scaling and root planning using universal curette and ultrasonic instrument in four sessions and instructed for routine oral hygiene measures. All patients re-evaluated 4 weeks after initial treatment, twenty patients of both sexes with at least two contralateral furcation grade II defects in a split mouth design were classified into 2 groups as follow:

Group I: Patients treated with cerabone® bone grafting material alone.

Group II: Patients treated with (cerabone®) plus PRF membrane.

Surgical steps: (Fig. 1)

The following procedures were done the same to the both groups

1- Buccal and lingual sulcular incisions were made after Surgical site was anaesthetized by block anesthesia, and mucoperiosteal flaps were reflected extended one tooth mesially and distally to provide visibility and accessibility to the defect.

2- Full thickness mucoperiosteal flap was raised and thorough open flap debridement was done.

3- Meticulous furcation defect debridement and root planing were performed using ultrasonic
instruments and area specific curettes. No osseous recontouring was performed.

4- The surgical area was irrigated with normal saline and carefully inspected for any remaining granulation tissue or deposits. Any adherent granulation tissue was trimmed from flaps.

5- **For Group I:** cerabone® were mixed with saline according to the manufacture instructions, and was applied onto the furcation defects with a sterile instrument.

6- **For Group II:**

- **PRF preparation** (Fig. 2): A blood sample was taken without anticoagulant in 10 ml tubes and immediately centrifuged at 3000 rpm for 10 minutes. The resultant product consists of following three layers: (a) RBC at the bottom, (b) PRF clot in middle and (c) upper most layer consisting of platelet poor plasma (PPP). PRF was easily separated from red corpuscles base (preserving a small red blood cell layer) using a sterile tweezers.

- Driving out the fluids trapped in the fibrin matrix by squeezing the PRF clot between sterile dry gauze, and autologous PRF membrane was obtained [9].

- Cerabone® were mixed with saline and applied in the same way as in group I. A compressed PRF membrane was trimmed and adapted over the grafted defect.

7- The mucoperiosteal flaps were repositioned and secured in place using 5-0 absorbable vicryl surgical sutures. Interrupted sutures were placed, and the surgical area was protected and covered with non-eugenol periodontal dressing.

8- Antibiotics (Augmentin ®) (GlaxoSmithKline, Egypt) (“Amoxicillin + clavulenic acid” 1gm twice daily for 7 days), and anti-inflammatory: 400 mg ibuprofen (Brufen ®, Abbott, Egypt), three times daily as necessary were prescribed, along with chlorhexidine digluconate rinses (0.12%) twice daily for 2 weeks were prescribed for all patients.

**Fig (1) Surgical steps A) surgical exposure of the furcation, B) The PRF membrane, C) cerabone®, D) application of PRF membrane in, E) application of cerabone®.**

**Fig (2) PRF preparation A) The three layers formed during PRF preparation, B) removal of the fibrin clot using sterile dry gauze, C) The PRF membrane.**
9- Periodontal dressing and sutures were removed 1 weeks postoperatively.

**(IV) Assessment method:** - Both groups were evaluated clinically and radiographically at regular basis at baseline (prior to surgery) and 6 months post-operative. Every patient was assessed by the following the clinical parameters, gingival index (GI) by **Loe and Silness in 1963** \(^{(43)}\), plaque index (PI) by **Silness and Loe in 1964** \(^{(44)}\), vertical clinical attachment level (VCAL) and Horizontal clinical attachment level (HCAL). While radiographic evaluation using Cone-beam Computed Tomography (CBCT) include measurement of: (fig. 3, 4)

i. Furcation Height (FH): Measured from the furcation fornix to the base of the alveolar base
ii. Furcation Width (FW): Measured between the greatest dimensions of separation between the two roots above the crest of alveolar bone
iii. Furcation Depth (FD): Measured from the crest of alveolar bone till the interradicular bone.

- Scans were obtained using SCANORA® 3Dx CBCT dental unit and the images were visualized using the scanner’s native software (SCANORA® 3Dx produces image data in DICOm®* format) On-Demand 3d software.

**RESULTS**

Twenty patients with a total number of 40 grade II furcation defects in split mouth design determined clinically and radiographically were selected for the study. The selected sites were treated with open flap surgery and were divided into two groups: Group I was treated with cerabone® only and Group II treated with cerabone® plus PRF membrane. Wound healing was normal with neither infectious episodes nor unpleasant clinical symptoms for both groups.

Regarding the clinical parameters, both groups showed significant improvement from baseline to the end of the study. However, only HCAL showed significant improvement in group II at 6 months when compared to group I. Changes in the radiographic parameters in each group were illustrated in tables 1 and 2, and when comparing the radiographic of Group I with Group II at 6 months, there was significant improvement in FH, FW, FD. Comparison of the clinical and radiographic parameters between the two groups at baseline and after 6 months after treatment were illustrated in table 3.
TABLE (1): Comparison between the radiographic measurements before and after treatment in group I

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height at baseline</td>
<td>2.22±0.66</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 months after surgery</td>
<td>1.40±0.42</td>
<td></td>
</tr>
<tr>
<td>Width at baseline</td>
<td>1.80±0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 months after surgery</td>
<td>1.06±0.37</td>
<td></td>
</tr>
<tr>
<td>Depth at baseline</td>
<td>2.44±0.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 months after surgery</td>
<td>1.32±0.26</td>
<td></td>
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</tbody>
</table>

*p value < 0.05 (significant)*

*p value > 0.05 (not significant)*

TABLE (2): Comparison between the radiographic measurements before and after treatment in group II

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height at baseline</td>
<td>2.32±0.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 months after surgery</td>
<td>1.13±0.27</td>
<td></td>
</tr>
<tr>
<td>Width at baseline</td>
<td>1.90±0.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 months after surgery</td>
<td>0.87±0.27</td>
<td></td>
</tr>
<tr>
<td>Depth at baseline</td>
<td>2.55±0.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 months after surgery</td>
<td>1.07±0.32</td>
<td></td>
</tr>
</tbody>
</table>

*p value < 0.05 (significant)*

*p value > 0.05 (not significant)*

TABLE (3) Comparison of the clinical and radiographic parameters between the two groups at baseline and after 6 months after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Interval</th>
<th>G I</th>
<th>G II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>Baseline</td>
<td>1.82±0.32</td>
<td>1.62±0.43</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>0.94±0.34</td>
<td>0.88±0.49</td>
<td>0.4</td>
</tr>
<tr>
<td>PI</td>
<td>Baseline</td>
<td>1.71±0.56</td>
<td>1.91±0.19</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>0.51±0.56</td>
<td>0.69±0.56</td>
<td>0.08</td>
</tr>
<tr>
<td>VCAL</td>
<td>Baseline</td>
<td>2.85±1.18</td>
<td>2.76±0.96</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>1.32±0.68</td>
<td>1.12±0.51</td>
<td>0.06</td>
</tr>
<tr>
<td>HCAL</td>
<td>Baseline</td>
<td>3.19±0.79</td>
<td>3.25±0.96</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>1.67±0.64</td>
<td>1.17±0.59</td>
<td>0.001*</td>
</tr>
<tr>
<td>FH</td>
<td>Baseline</td>
<td>2.22±0.66</td>
<td>2.32±0.65</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>1.40±0.42</td>
<td>1.13±0.27</td>
<td>0.02*</td>
</tr>
<tr>
<td>FW</td>
<td>Baseline</td>
<td>1.80±0.45</td>
<td>1.90±0.43</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>1.06±0.37</td>
<td>0.87±0.27</td>
<td>0.04*</td>
</tr>
<tr>
<td>FD</td>
<td>Baseline</td>
<td>2.44±0.51</td>
<td>2.55±0.57</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>1.32±0.26</td>
<td>1.07±0.32</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

*p value < 0.05 (significant)*

*p value > 0.05 (not significant)*
DISCUSSION

Furcation involvement is one of the most common dentoalveolar squeal of periodontitis, and its management presents an exclusive clinical problem in periodontal therapy. Numerous studies have confirmed that furcation involved molars respond less favorably to periodontal therapy than molars without furcation involvement or single-rooted teeth, and are at a higher rate of periodontal breakdown than other teeth [20].

The present study was designed as a split-mouth investigation to facilitate the comparison of two regenerative techniques by eliminating patient-specific characteristics that might have an impact on the results of regenerative surgeries. This design minimizes any human variables that influence the human healing power, immune response, plaque control effectiveness, and recall frequency [21].

There are a little studies on role of PRF in management of furcation defects [22-24]. So, the present study was designed to investigate the using of cerabone® alone or in combination with PRF to evaluate regenerative process of Class II furcation defects.

Autologous PRF is a recent and promising innovation in regenerative periodontal therapy. PRF is an organized dense fibrin scaffold with a specific slow release of growth factors such as TGF-β1, PDGF-AB, and VEGF and glycoproteins such as thrombospondin [25].
PRF also induces the proliferation of various cells in vitro with the strongest induction effect on osteoblasts. PRF in combination with various bone graft promoting wound healing, bone growth maturation, graft stabilization, hemostasis, and reduces healing time [26,27]. In osteoblasts cultures: PRF was proven to upregulate phosphorylated extracellular signal-regulated protein kinase expression and suppress osteoclastogenesis by promoting the secretion of osteoprotegerin and alkaline phosphatase in osteoblasts cultures [28].

Cerabone® is characterized by rapid and complete hydration with blood or saline solution which is crucial for excellent handling characteristics, new bone formation and for the final clinical success. Its strong capillary action facilitates fast and efficient penetration of the material particles with fluids, nutrients and blood through the three dimensional, porous trabecular bone network, resulting in excellent handling and reliability in the daily clinical use [29].

The rough surface also promotes the adhesion of serum proteins and cells onto the surface. Osteoblast-like cells quickly adhere to the cerabone® particles. Only attached osteoblasts can start to produce new bone matrix leading to the osseous integration of the cerabone® particles. Another study indicated that the good adherence of osteoclasts promotes the superficial remodeling of the particles [30].

In our study we opted for CBCT as the radiographic assessment tool owing to its advantages regarding accuracy, lower radiation and higher reproducibility, and also based on work done by Grimard et al., 2009 who compared clinical, periapical, radiograph and CBCT measurement techniques for assessing bone level changes following regenerative therapy in periodontal intrabony defects, and concluded that CBCT was significantly more precise and accurate than intraoral radiographs [31].

In the current study, both treatment modalities succeeded a statistically significant reduction in the mean plaque and gingival scores at the treated sites during follow-up evaluations compared with baseline scores. The main changes of plaque and gingival index scores indicated that the oral hygiene status of the patients who participated in the present work was good and that patient cooperation plays an important role in periodontal regeneration. These results were in agreement with several studies [32,33].

The mean gain in VCAL for individual groups showed statistically significant results over 6 months of the study. Gain in CAL was 1.32±0.68 mm in the Group I and in

Group II 1.12±0.51 ($P = 0.06$), which is statistically not significant post surgically. These observations is similar to the results of studies [32,33,34] revealed that statistically significant mean reduction in vertical probing depths and gain in vertical clinical attachment levels and linear bone fill. This is in agreement with the study of Fleischer et al. [35], they observed that open root planing left the affected area more free from calculus depositions, as compared to the closed debridement technique.

Significant improvement in HCAL clinically and FH, FW, FD radiographically for both groups to baseline may be due to the natural bone surface structure of cerabone® (micro- and macro pores, rough surface). Cerabone® is a highly porous bone grafting material with a porosity of ~65-80% and a mean pore size of ~600-900 μm. Macro pores enable a fast ingrowth of blood vessels and bone cells, while micro pores support fast blood uptake by the capillary effect [36]. Bone formation starts early following implantation (3-6 weeks). During the formation of a blood clot, different cell types involved in the wound healing cascade and growth factors originating from the wound area bind to the surface of the scaffold. Precursor cells differentiate into osteoblasts and start to produce new bone matrix. After a few months, the cerabone® particles are integrated into the newly formed bone matrix and the surface is completely covered by new mineralized bone resulting in a long-term stable bone situation [37,38].
The results of the present study showed that treatment of furcation defects with both Cerabone® plus PRF (group II) membrane leads to significant reduction in HCAL clinically and FH, FW, FD radiographically as compared to Cerabone® alone (group I) at 6 months. The mean values of HCAL in group I and group II were 1.67±0.64 and 1.17±0.59 respectively (p =0.001). The mean reductions of FH, FW and FD in group I at 6 months were 1.40±0.42, 1.06±0.37 and 1.32±0.26 while in group II were 1.13±0.27, 0.87±0.27 and 1.07±0.32 respectively. The results revealed that significant differences between the two groups at 6 months. So, it indicated that the combination of PRF with Cerabone® increased the regenerative process of alveolar bone. The positive impact of PRF on bone healing could be attributed to the angiogenic, proliferative and differentiating effects on osteoblasts of tissue growth factor β and platelet derived growth factor (TGF-β and PDGF) that are present in PRF in high concentrations [39].

In accordance with our results, Eldibany et al., 2014 suggested that the combination of bone grafts along with the growth factors in the PRF may be suitable to enhance the bone density [40]. The Platelet-rich fibrin (PRF) can be used in conjunction with bone grafts, which offers several advantages including promoting wound healing, bone growth and maturation, graft stabilization, wound sealing, and hemostasis and improving the handling properties of graft materials [41]. Sharma and Pradeep (2011) stated that PRF has been shown to be an effective modality of therapy in the regenerative treatment of degree II mandibular furcation [34].

CONCLUSION

Both Cerabone® and Cerabone® plus PRF significantly improved the clinical and radiographic parameters at 6 months interval. The adjunctive use of PRF resulted in clinically, radiographically and statistically significant differences compared with Cerabone® bone graft alone, in terms of HCAL, FH, FW and FD.

REFERENCES

10. Charrier, J., et al.,(2008) Relevance of Choukroun’s Platelet-Rich Fibrin (PRF) and SMAS flap in primary


