A Novel Surgical Approach for Treatment of Class II Furcation Defects Using Marginal Periosteal Membrane

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Abstract

Objectives: This study was designed to describe and evaluate the use of a vascularized marginal periosteal barrier membrane (MPM) harvested by a semilunar incision, alone or combined with a bone graft, in treatment of class II furcation defects in mandibular molars, compared to open flap debridement (OFD).

Methods: Thirty class II furcation defects in mandibular molars were randomly assigned into three equal groups: Group I included OFD, Group II included defects treated with MPM, and Group III consisted of defects treated with MPM after applying demineralized freeze-dried bone allograft (DFDBA). At baseline and 6-month follow-up, vertical probing depth (VPD), clinical attachment level (CAL) measurements, along with a radiographic measurement of bone height (BH), were obtained for each defect. Transmission electron microscopy (TEM) was used for further evaluation of the histological changes associated with gingival samples related to each line of treatment.

Results: Both Groups II and III reflected significant favorable outcomes in all the assessed parameters compared to OFD. A non-significant difference was found between both groups regarding VPD, while significant improvement in CAL and BH were detected in Group III ($p \leq 0.05$). Favorable histological findings were also noticed in the test groups, with more improvement in Group III.

Conclusion: Placement of a vascularized MPM as a barrier membrane, using a semilunar incision, demonstrated a significant improvement in both clinical and histological outcomes of class II furcation defects in lower molars. When it was combined with DFDBA, a meaningful difference was found with regard to early wound healing and gain in CAL and BH.

Keywords: Periosteal autogenous membrane, class II furcation defects, demineralized freeze-dried bone allograft, guided tissue regeneration.

Introduction

Treatment of furcation lesions is one of the most challenging tasks in periodontal therapy. The furcal anatomical features (e.g., small ridges, peaks and pits forming convexities and concavities) offer limited access for routine periodontal debridement. The presence of accessory canals in the furcation region in up to 25% of permanent molars, as well as the presence of enamel projections in 29% of mandibular and 17% of maxillary molars, further complicates furcation management (Pradeep *et al.*, 2009).

In class II furcation involvement, the treatment is more uncertain. Although it was shown that this type of defect could be successfully treated by regeneration, the predictability related to the treatment type remains a major issue. Therefore, proceeding with caution is definitely advised (Avila *et al.*, 2009). It should be remembered that spontaneous and predictable regeneration following meticulous debridement is possible, especially with the availability of bone morphogenetic proteins (BMPs) and enamel matrix derivatives (Sánchez-Pérez and Moya-Villaescusa, 2009).

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However, it might be inadequate for the treatment of deep pockets or wide circumferential furcation defects (Verma *et al.*, 2013).

Tunneling has been proposed as a conservative alternative in cases of furcation class II. However, long-term survival is not ensured, as many complications (among which root caries predominates) may arise after treatment, compromising tooth prognosis (Avila *et al.*, 2009). Root resection is a conservative therapeutic option indicated in some furcation defects to provide a better environment and maintain the tooth. Although it was shown that root-resected teeth have good long-term survival rates, previous studies (Langer *et al.*, 1981; Buhler, 1988), showed that root-resected teeth had survival rates of 85% and 68% after 5 and 10 years, respectively. Therefore, it can be acknowledged that a root-resected tooth has less periodontal support and a less favorable prognosis than a healthy one (Avila *et al.*, 2009).

To increase the predictability and clinical success of regenerative therapy, factors related to the patient, furcation, surgical treatment, and postoperative period should be also considered. The rationale for guided tissue regeneration is to exclude gingival epithelium and connective tissue from the alveolar bone and root surfaces by placing a physical barrier, thus creating areas into which progenitor cells from the periodontal ligament and alveolar bone can migrate. Guided tissue regeneration has many indications in periodontal therapy, among the most important being treatment of class II furcation lesions. However, the resulting improvements are modest and variable. The revascularization of any flap may be further compromised by blockage of the potential blood supply from the periodontal ligament and bone defect to the connective tissue flap by a membrane (Novaes *et al.*, 2005). Perhaps the most important factor affecting guided tissue regeneration outcome is the periosteal isolation (Gamal and Iacono, 2013).

Periosteum is an attractive alternative to the existing barrier techniques, because it is biologically accepted. The utilization of an autogenous marginal periosteal membrane (MPM) apical to the furcation lesion provides several clinical benefits, among which is that a second graft donor site is not required. The vitality of the barrier may be ensured by a proper blood supply from the base of the mucoperiosteal flap, and possibly the gingival flap itself, which may further modify the cellular dynamics of the wound in favor of clinical outcome. The supposed physiologic mode of growth factors delivery that could be achieved by periosteal osteoprogenitor cells offers an advantage to the use of MPM as a biologic barrier, representing a promising way to get a more predictable amount of periodontal regeneration (PR; Gamal *et al.*, 2010).

Periosteum is a highly vascular connective tissue comprised of two distinct layers: a thick, outer non-osteogenic fibrous layer and a thin inner cellular osteogenic-cambium layer. It was reported that reverting the periosteum such that the fibrous layer is in contact with the defect provided reliable results in the treatment of class II furcations. However, histological findings have questioned the cellular differentiation in the defect area, as bone-forming cells migrated from the inner to the outer layer of periosteum during healing (Amicarelli and Alonso, 1999). Hence, it is preferable to find a technique that directly uses the inner cambium, known to have the potential to stimulate bone formation (Ito *et al.*, 2001).

In 2003, Murphy and Gunsolley concluded that use of augmentation materials with physical barriers enhances the regenerative outcome in furcation treatment. They function as a scaffold to ensure clot stabilization and to provide a space for regeneration. Allogeneic or autogenous materials were used with no significant differences. However, because of the limited amount of intraoral donor bone, it is preferable to use demineralized freeze-dried bone allograft (DFDBA) in large defects (Abolfazli *et al.,* 2008).

Therefore, this study was designed in an attempt to describe and evaluate the use of a vascularized MPM, harvested by the semilunar incision, in treatment of mandibular class II buccal furcation defects. Clinical and histological assessments were undertaken to evaluate MPM, with and without DFDBA, versus open flap debridement (OFD).

Materials and methods

This study was divided into two parts: the first part dealt with the clinical efficacy of MPM in treatment of class II furcation defects in chronic periodontitis patients, either alone or combined with DFDBA, versus OFD. The second was a qualitative part aimed at evaluating the potential induced structural changes of the gingiva with each line of treatment.

Pre-surgical therapy and patient selection

Twenty-six subjects (15 women and 11 men) participated in the study, ranging in age from 37 to 52 years (mean = 42.6), diagnosed with chronic periodontitis according to clinical and radiographic findings (Armitage, 1999). They were selected from the outpatient clinic of the Department of Periodontology, Faculty of Dental Medicine, Al Azhar University (Girls Branch).

Inclusion criteria

Patients were screened for the following criteria: 1) the presence of a class II buccal furcation defect in a mandibular molar according to Glickman's classification (1953); 2) no systemic diseases that could influence

the outcome of the therapy, as evaluated by modified Cornell medical index (Abramson, 1996); 3) good compliance with plaque control instructions following initial therapy, using the plaque index values (0 or 1) according to Silness and Löe (1964); 4) vertical probing pocket depths (VPD) of ≥ 5 mm and clinical attachment levels (CAL) ≥ 4 mm four weeks following initial therapy; 5) gingival margin positioned coronally to the furcation fornix. Each subject had undergone initial periodontal therapy without occlusal adjustment (as such adjustment was not indicated in any of the cases).

Exclusion criteria

Smoking patients, former smokers, pregnant and lactating females, and previously treated subjects, along with those taking oral contraceptives, were excluded from participating in this study. Patients were also excluded if they presented with inadequate compliance with the oral hygiene maintenance schedule.

Before acceptance into the study, each patient received a brief description of the investigation and provided signed informed consent. The Ethical Committee of Al Azhar University approved the experimental protocol.

Patient grouping

All participants were re-evaluated 4 weeks following the initial therapy to confirm the need for regenerative furcation reconstructive surgery for the selected sites (Nejad *et al.,* 2004). A total of 30 defects were randomly assigned, using a computer-generated table, to one of the following equal treatment groups (n = 10 defects/group) in a parallel, double-masked design. Group I (control) included defects treated with OFD, Group II (Test 1) included defects treated with MPM, and Group III (Test 2) included defects treated with MPM plus DFDBA.

Clinical parameters

Baseline data for all sites were collected just prior to the surgical phase of treatment. Soft tissue measurements included VPD and CAL measurements using a calibrated periodontal probe with William's markings to the nearest millimeter as the distance from the base of the pocket to the gingival margin and the cemento-enamel junction (CEJ), respectively. Measurements were recorded at three sites for each tooth: mesio-buccal line angle, disto-buccal line angle and mid-buccal line angle. The mean of the three readings was taken. The precise position of the periodontal probe was recorded using custom-made acrylic occlusal stents.

A computer program (Dür DBS Win image processing software) for image processing and manipulation was used to evaluate the linear measurements related to the changes in each defect bone level from routine diagnostic standardized periapical views, using the long cone/paralleling technique and aiming devices. All radiographs were digitized using a flatbed scanner with a scanning resolution of 600 dpi (UMAX-A8TRA 12208). In each digitized radiograph, three lines were drawn from the furcation roof to the base of each defect, representing the defect bone height (BH), and the means were calculated.

Clinical and radiographic measurements (*Figures 1a, 1b*) were re-assessed six months after therapy by one calibrated masked examiner to evaluate the quantitative changes in each defect. Intra-examiner reproducibility was assessed with a calibration exercise performed on two separate occasions 48 hours apart. Calibration was accepted if ≥ 90% of the recordings could be reproduced within a difference of 1.0 mm.

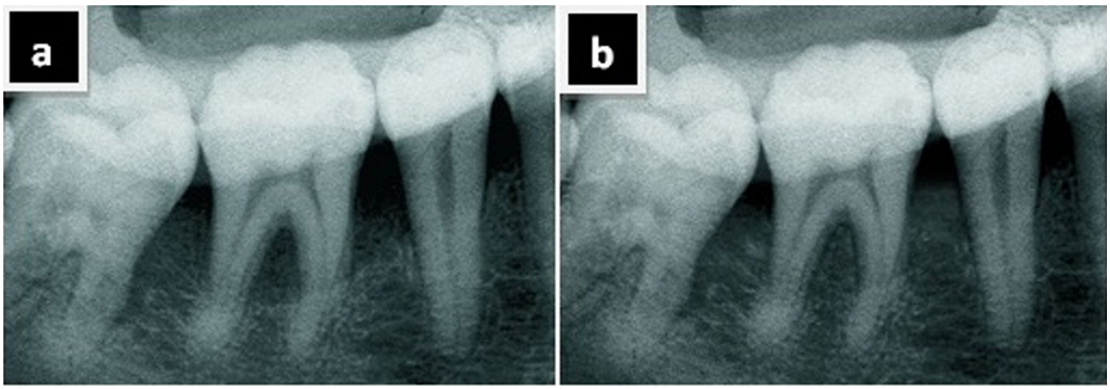

Figure 1. a) A preoperative periapical radiograph of the class II furcation defect; b) A periapical radiograph of the same site taken at six months.

Surgical protocol

One surgeon performed all surgeries. For Groups II and III, following administration of a local anesthetic agent (lidocaine 2% with epinephrine 1:80,000), two vertical releasing incisions that extended into the alveolar mucosa were placed no closer than one tooth mesial and distal to the selected tooth. An intrasulcular incision was then performed to join the vertical incisions. A partial thickness flap was raised at the buccal aspect of the tooth using a Bard-Parker blade No. 11. Granulation tissue was removed, and root surfaces were thoroughly scaled and planed with hand and rotary instruments. A high-speed finishing bur was used to remove any enamel projections. Following the dissection, periosteum remained on bone and a partial thickness of the gingival connective tissue remained on the periosteum. A semilunar incision was made to fenestrate the periosteum 3-4 mm apical to the defect osseous margin, excluding a sufficient mucoperiosteal insertion zone coronal to the incision (*Figures 2a, 2b*); followed by careful elevation of periosteum using a mucoperiosteal elevator (*Figures 3a, 3b*).

In Group III, furcation defects were filled with DFDBA (Suross™ manufactured by Hans Biomed Corp., Sungdong-gu, Seoul, Korea). The periosteum was then coronally moved to seal the furcation lesion such that the cambium layer was juxtaposed to the exposed furcation, (*Figure 4a,b*). Using a sling-suture technique, a bioresorbable ligature was used to tightly secure the coronal portion of the periosteum to the tooth. Finally, the flap was coronally repositioned and secured with interrupted sutures using 4-0 black silk suture. In Group I, full thickness mucoperiosteal flaps were elevated, then replaced after defect debridement and secured with interrupted suturing.

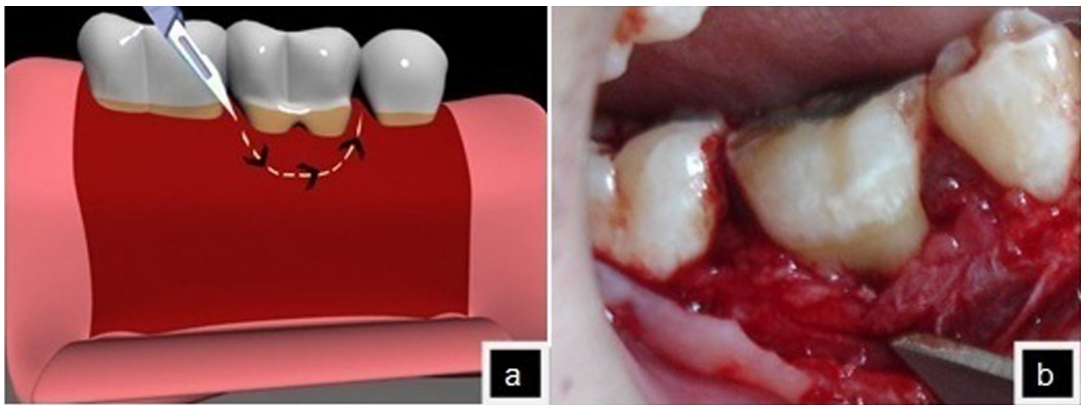

Figure 2. a) A diagram showing the periosteal barrier semilunar incision design. Notice that the design excludes a mucoperiosteal insertion zone coronal to the osseous margin. b) A clinical photograph showing the semilunar incision using Bard-Parker blade No. 11 to fenestrate the periosteum 3-4 mm apical to the osseous margin of the defect, excluding a sufficient mucoperiosteal insertion zone coronal to the osseous margin to ensure blood irrigation.

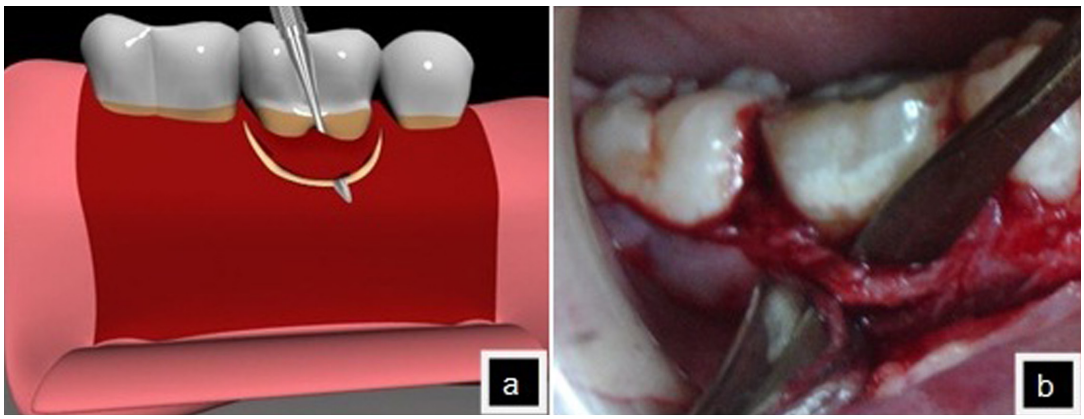

Figure 3. a) A diagram showing the elevation of the periosteum using the mucoperiosteal elevator; b) A clinical photograph showing the careful elevation of periosteum using the mucoperiosteal elevator.

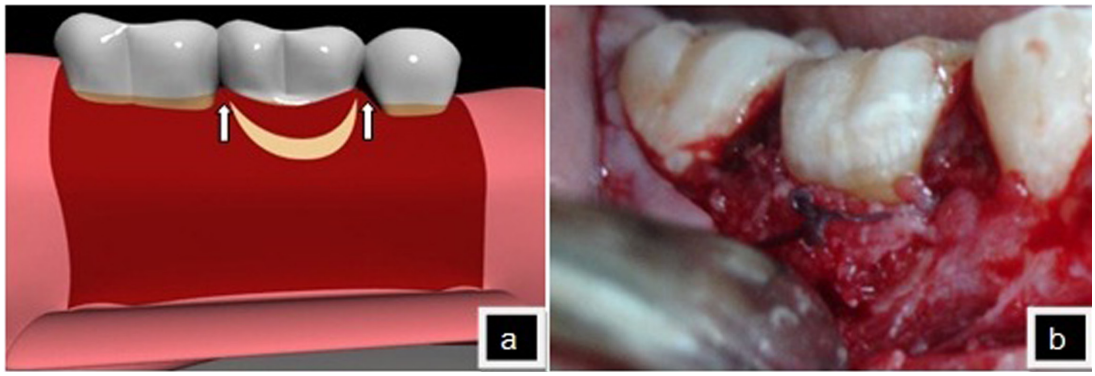

Figure 4. a) A diagram showing the coronal direction of mobilizing the periosteum (the white arrows), to seal the defect such that the cambium layer is juxtaposed to the exposed furcation; b) A clinical photograph showing the coronal mobilization of the periosteum.

Post-operative care

A platinum foil was used to cover the operation site, followed by a perio-pack. Removal of sutures was done 14 days after surgery. Analgesics (400 mg ibuprofen) were used for patients experiencing post-surgical discomfort. Patients were placed on an infection control program including seven days of systemic antibiotic therapy (amoxicillin-clavulanic acid, 2 gm/day) and 6 weeks of chemical plaque control (0.12 % chlorhexidine gluconate rinses, twice daily).

Patients were instructed to forego mechanical plaque control 2 weeks post-operatively. Oral hygiene procedures were then gently initiated in the surgical areas with a soft brush. Professional prophylaxis for plaque removal was performed at weekly intervals for 4 to 6 weeks, then monthly until the end of the study.

Histological study

Gingival specimens were taken from chronic peritonitis patients (7 samples in each group) who had buccal furcation-affected mandibular molars diagnosed as hopeless for dental-periodontal reasons and designated for extraction. Each of the furcation defects was subjected to OFD, MPM or MPM+DFDBA. On day 10 after surgery, teeth were extracted and gingival biopsy specimens were obtained full length along the dento-gingival region and immediately above the alveolar crest (Lafzi *et al.*, 2007). The gingival tissues were carefully dissected and cut into 1 x 1 mm cubes, then placed in labeled jars for histological evaluation.

Preparation for transmission electron microscopic examination (TEM)

Specimens for TEM examination were prepared according to Bancroft and Stevens (1982). Fixation was done by immersion of the specimens in a mixture of 2.5% glutaraldehyde and 10% formaldehyde (F/G solution) for 24- 48 hrs (Dard *et al.,* 1989). Then, specimens were washed several times in phosphate buffer solution with pH 7.2 – 7.4, post-fixed in 1% osmium tetroxide for 1 hr, and washed again in phosphate buffer. The specimens were passed through ascending concentrations of ethyl alcohol for dehydration. They were then infiltrated and embedded in flat rubber molds filled with embedding resin (Epon 812). After polymerization, the blocks were cut into semi-thin sections (1 μm) with a glass knife via ultramicrotomy. Sections were then mounted on glass slides and stained with 1% toluidine blue. Certain areas of interest were selected for ultrathin sectioning. Ultrathin sections were obtained at 60 nm using a diamond knife and placed/collected on a grid of copper. Grids were stained with uranylacetate. The grids, with the specimen side down, remained in 4% uranyl acetate for 25 minutes and were then rinsed in a series of four beakers of pure water. After rinsing, the grids were then stained with 1% lead citrate for 5 minutes and rinsed again in pure water. The specimens were examined with a JEOL-Japan 1010 TEM and photographed using an AMT XR40 digital camera with 2k x 2k pixels at the Regional Center for Mycology and Biotechnology (RCMB)-Al Azhar University.

A single examiner performed examination of TEM micrographs after masking the group number to make the evaluation unbiased.

Statistical analysis

Clinical data were presented as mean and standard deviation (SD) values. Kruskal-Wallis test was used to compare between the three groups. Mann-Whitney U test was used in the pair-wise comparisons between the groups. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 16.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

Results

Clinical parameters

All patients completed treatment and post-treatment phases successfully, and healing was satisfactory. Before treatment, there was no statistically significant difference between the three groups in all parameters. Changes in PD, CAL and BH are shown in *Table 1*.

At 6 months postoperatively, the mean percentage of reduction in PD in Group I was 28.7% ± 18.3, 60% ± 8.6 in Group II, and 57.3% ± 6.8 in Group III ($p < 0.001$), respectively. At the end of the study, the statistically significantly lowest percentage reduction was shown in Group I, while a non-significant difference was found between Groups II and III.

The mean percentage of reduction in CAL in Groups I, II and III at 6 months was 29.0% ± 23, 63.7% ± 8.8 and 77% ± 13.6, respectively ($p < 0.001$). Concerning the post-operative mean percentage of changes in BH, Groups I, II and III showed a reduction of 11.9% ± 10.8, 59.7% ± 13.4 and 67.2% ± 8.5 at 6 months respectively, ($p < 0.001$). Group III showed the statistically significantly highest mean percentage reduction both in CAL and BH, followed by Group II.

Descriptive histology

The epithelial phase

For Group I, severe ultra-structural alterations were detected, while the orientation of epithelial cell layers was more or less clear. The most common feature was represented by vacuolization, edema and interruption of intercellular cell junctions. The basal lamina was obviously corrugated and interrupted in definite small areas along its course. The basal epithelial cell membranes appeared irregular and severely corrugated. The cytoplasmic organelles showed severe vacuolization and hyalinization. The intermediate cells appeared compressed with wide extracellular matrix (ECM). Superficially, the cells were very pyknotic and no keratin flakes were detected (*Figure 5a*). However, enhanced ultra-structural features were noticed in Group II. The cellular membranes were well defined but irregular. The cytoplasmic components showed moderate degrees of hyalinization and vacuolization (*Figure 5b*). In Group III, more favorable features were reflected. The epithelial cell layers had a regular shape and orientation along the different areas of gingival epithelium. Their cell membranes showed clear, regular and smooth outlines. Their nuclei were markedly open-faced with regular nuclear membranes and chromatin distribution. The intercellular junctions, mostly desmosomes, were numerous and dominant, especially between the basally situated cells, with better configuration (*Figure 5c*).

The connective tissue phase

In Group I, fibroblasts appeared shrunken and ovoid, with marked reduction in cytoplasmic and nuclear volume and short cytoplasmic extensions. The cytoplasm appeared hyalinized with poorly defined cytoplasmic organelles. Dilated and irregular nuclear membranes with chromatin clumping were observed. The intercellular junctions between the fibroblastic cell processes were completely lost. The ECM expressed dispersed areas of hyalinization, vacuolizations and dilated blood vessels. Some transversal and longitudinal collagen fibers were seen extra-cellularly (*Figure 6a*). In Group II, fibroblasts preserved their spindle shape with normal cytoplasmic process. Numerous longitudinal and transverse collagen fibers were proliferating among the ECM and in close proximity to fibroblasts (*Figure 6b*). In Group III, fibroblasts possessed favorable morphology with normal cytoplasmic processes and organelles, such as prominent amounts of rough endoplasmic reticulum and mitochondria. Densely packed collagen bundles were detected close to the fibroblastic cell processes (*Figure 6c*).

Table 1. The means, standard deviation (SD) values and results of comparison among the three groups.

Variable	Time	Group I			Group II			Group III			*p* value
		Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	
VPD	Baseline	4	0.9	0.3	3.6	1.4	0.5	3.8	1.2	0.4	0.668
	6 months	2.8[a]	0.8	0.2	1.4[b]	0.5	0.2	1.6[b]	0.5	0.2	0.001*
	% reduction	28.7[b]	18.3	5.8	60[a]	8.6	2.7	57.3[a]	6.8	2.2	<0.001*
CAL	Baseline	3.8	0.8	0.2	3.8	0.8	0.2	4	0.7	0.2	0.748
	6 months	2.8[a]	1.2	0.4	1.3[b]	0.5	0.2	1[b]	0.7	0.2	0.003*
	% reduction	29[c]	23	7.3	63.7[b]	8.8	2.8	77[a]	13.6	4.3	<0.001*
BH	Baseline	5.4	1.1	0.3	5	0.9	0.3	5.8	0.8	0.2	0.215
	6 months	4.8[a]	1.2	0.4	2[b]	0.7	0.2	1.9[b]	0.4	0.2	<0.001*
	% reduction	11.9[c]	10.8	3.4	59.7[b]	13.4	4.3	67.2[a]	8.5	2.7	<0.001*

*Significant at $p \leq 0.05$. Means with different letters (a, b and c) are statistically significantly different. VPD, vertical probing depth; CAL, clinical attachment level; BH, bone height

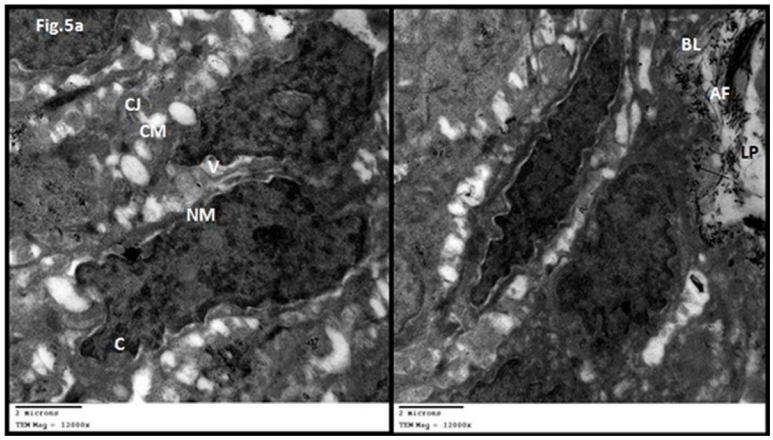

Figure 5a. An electron micrograph of Group I (open flap debridement only) showing basal epithelial cells with irregular cell membrane (CM), irregular nuclear membrane (NM), chromatin clumping (C), vacuolized cytoplasm (V), vacuolized intercellular cell junctions (CJ), basal lamina (BL) interrupted (arrow), anchoring fibers (AF), lamina propria (LP). Original magnification 12000X.

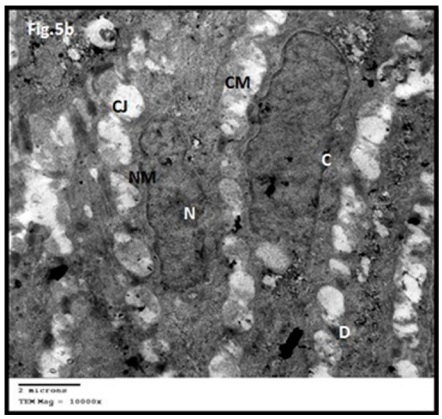

Figure 5b. An electron micrograph of Group II (open flap debridement plus marginal periosteal barrier membrane) showing basal epithelial cells, cell membrane (CM), enhanced nuclear membrane (NM), peripheral chromatin (C), central nucleolus (N), vacuolized intercellular cell junctions (CJ), desmosomes (D). Original magnification 10000X.

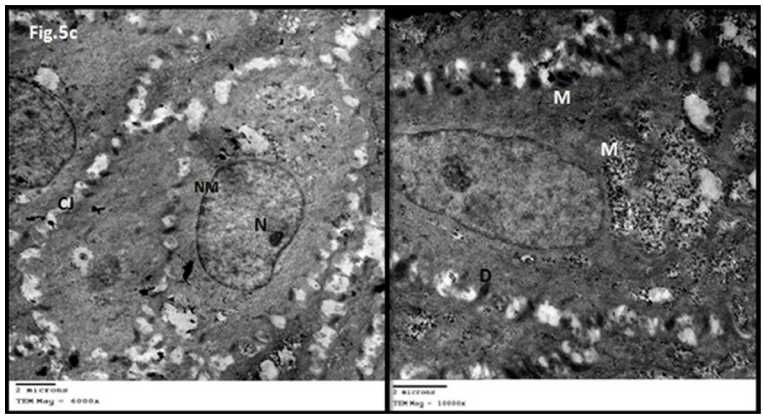

Figure 5c. An electron micrograph of Group III (open flap debridement plus marginal periosteal barrier membrane after applying demineralized freeze-dried bone allograft) showing basal epithelial cells with regular smooth nuclear membrane (NM), open-faced central nucleus (N), less vacuolized intercellular cell junctions (CJ), desmosomes (D) and swollen mitochondria (M). Original magnification 6000X and 10000X.

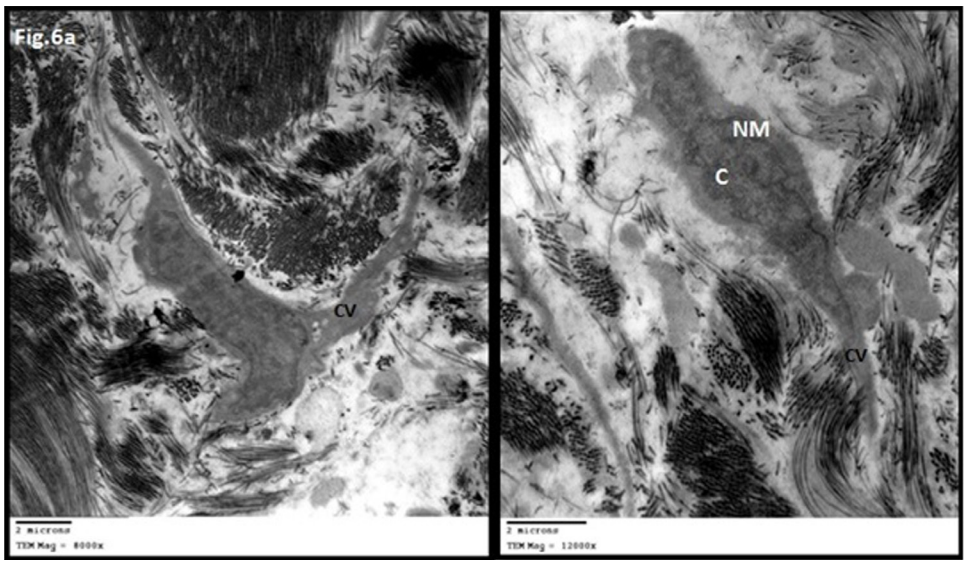

Figure 6a. An electron micrograph of Group I (open flap debridement only) showing fibroblastic cell with short cytoplasmic process, irregular nuclear membrane (NM), peripheral and central chromatin condensation (C), hyalinized reduced cytoplasmic volume (CV). Original magnification 8000X and 12000X.

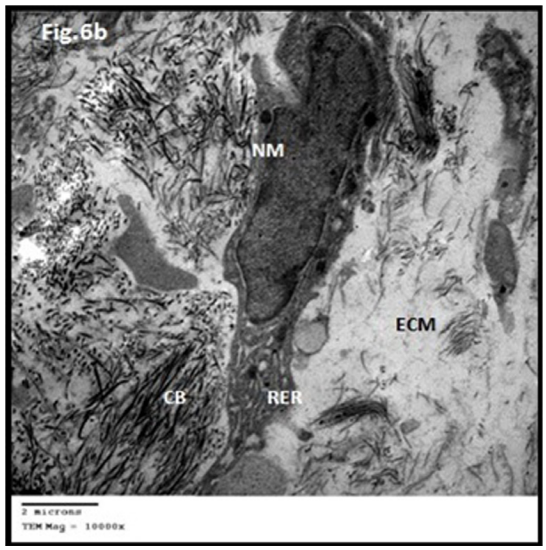

Figure 6b. An electron micrograph of Group II (open flap debridement plus marginal periosteal barrier membrane) showing spindle shaped fibroblastic cell with normal cytoplasmic process, irregular nuclear membrane (NM), swollen cytoplasmic organelles, prominent rough endoplasmic reticulum (RER), extracellular matrix (ECM), collagen bundles (CB). Original magnification 10000X.

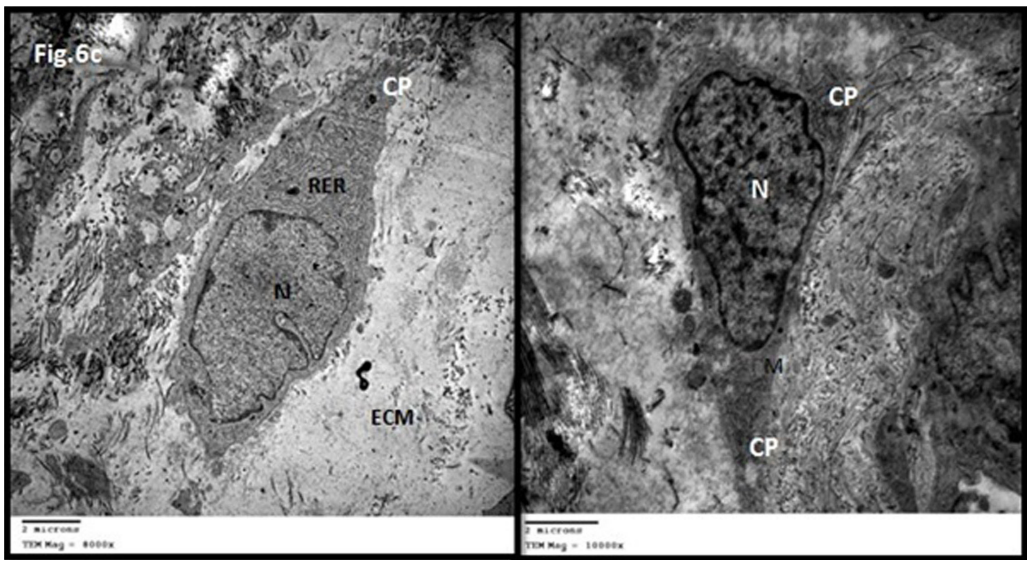

Figure 6c. An electron micrograph of Group III (open flap debridement plus marginal periosteal barrier membrane after applying demineralized freeze-dried bone allograft) showing fibroblastic cell with normal cytoplasmic process (CP), open faced nucleus (N), rough endoplasmic reticulum (RER), mitochondria (M), extracellular matrix (ECM). Original magnification 6000X and 10000X.

Discussion

The regeneration of class II furcation lesions, although possible, is not considered a totally predictable procedure, especially in terms of complete bone fill. Furcation morphology may restrict access for adequate debridement and may have a reduced source of available cells and blood supply from the periodontal ligament and bone defect (Novaes *et al.*, 2005).

There are several regenerative techniques, used alone or in combination, considered to achieve PR (Verma *et al.*, 2013). This study was conducted to describe and evaluate the use of MPM harvested for the first time by the semilunar incision, with and without DFDBA, for treating class II buccal furcation defects, compared to OFD.

Coronally positioned flaps may have the advantage of using the periosteum as a barrier membrane. However, if the PD is ≥ 5 mm in the furcation area, the flap tissue cannot be successfully coronally positioned. In the pocket area, a pathological transformation occurs of the flap tissue into a pocket wall; thus, the periosteum may get infected and destroyed with a defective regenerative ability. Therefore, periosteum displacement techniques are much preferred (Verma *et al.*, 2011). In terms of viability, the environment of the recipient site less affects a vascularized periosteum than a free periosteum. Besides, it does not require a second operation, and so there is less surgical trauma, fewer post-operative complications and better patient satisfaction (Krishna and Vijaya, 2012).

However, periosteum-harvesting techniques are usually accompanied by some technical difficulties such as the complexities of surgery, they are time consuming, and need a skillful operator to protect the membrane from laceration (Verma *et al.*, 2011). Importantly, it has been reported that bone formation is affected by the degree of surgical damage to the periosteum and the form of the periosteum while harvesting it (Cohen and Lacroix, 1955). In this respect, we can declare that our novel approach is simple and time saving, with careful handling of the MPM through blunt dissection. A recent immunohistochemical study was conducted to quantify human periosteal cells (Frey *et al.*, 2013). The authors reported that all stained cells were located in the inner cambium layer, and mostly consisted of stromal stem cells and osteoblastic precursor cells. Cells positive for markers of osteoblast, chondrocyte, and osteoclast lineages were also found. In the present study, an MPM harvested by the semilunar incision was coronally mobilized such that the cambium layer was juxtaposed to the exposed furcation. Accordingly, the cambium layer may directly work to initiate and drive the cell differentiation process of bone repair, as suggested by Grover *et al.* (2012).

Our approach seems to have an additional advantage related to the surgical stimulating effect secondary to the forceful displacement of the periosteum. In accord, Goldman and Smukler (1978) proposed the transfer of a pedicle flap with stimulated periosteum, using a needle before graft transplantation, to enhance the graft healing potential over the root. They further studied stimulated periosteal pedicle grafts in dogs, concluding that the healing of the stimulated flaps was accompanied by the formation of a relatively short dentogingival epithelium, cementogenesis, and new connective fiber insertion into cementum (Goldman *et al.,* 1983).

Regarding the clinical assessment, none of the investigated parameters in all study groups showed any statistical difference at baseline, thus ensuring the same starting point for all the tested procedures. The clinical parameters used in this study were chosen according to the 1989 World

Workshop in Clinical Periodontics. A 6-month follow-up period was chosen; although it may be too short to fully evaluate the effect of periodontal therapy, this seemed to be the standard time frame for this type of research (Hartman *et al*., 2004).

In Group II, the absolute values of the observed added benefits at the end of follow-up were 1.4 ± 0.5 mm for PD and 1.3 ± 0.5 mm for CAL. Our results confirm findings described by Gamal and Mailhot (2008), where clinical attachment gain and pocket resolution were achieved using marginal periosteal pedicle flaps and proved superior to treatment with OFD. Our results are also supported by the findings of Graziani *et al.* (2011), who reported that conservative surgery provides an additional benefit compared with closed debridement alone in treatment of intra-bony defects. However, the outcomes were generally inferior with respect to the regenerative techniques. Nevertheless, the costs of regenerative surgery are significant. In this context, our proposed approach is multi-advantageous; periosteum is safe, biologically accepted, highly regenerative (owing to the structural and molecular values of periosteum), and inexpensive (being a patient's own), in agreement with Gamal *et al.* (2010).

In this clinical trial, the quantification of bone fill by radiographic analysis was preferable to re-entry procedures, as well as more convenient for the patients. In addition, the new connective tissue attachment may be disturbed and replaced by a long junctional epithelium as a consequence of surgical re-entry. Crestal bone resorption may also occur as a result of the re-entry procedure (Sullivan *et al*., 2000).

In Group II, the observed added benefit was 2.0 ± 0.7 mm of BH; a statistically significant value compared to OFD. Similar results were reported by some investigators following application of MPM in mandibular class II furcation defects (Amicarelli and Alonso, 1999). This could be attributed to the periosteum potentiality to stimulate bone formation when used as a graft material (Mahajan, 2012). Other researchers have shed light on the critical roles of BMPs, fibroblast growth factor, platelet-derived growth factor and inflammation signaling in periosteal-mediated bone regeneration, fostering the path to novel approaches in bone-regenerative therapy (Lin *et al*., 2013).

Reviewing data published from studies carried out only on humans, the conclusions indicated that in furcation defects, better results are obtained with the combination of a bone grafting material plus a membrane (Alpiste-Illueca *et al*., 2006). Moreover, Bowers *et al.* (2003) concluded that a successful clinical closure of class II furcations was achievable following combination therapy with an expanded-polytetrafluoroethylene membrane and DFDBA. Furcations with vertical or horizontal bone loss of ≥ 5 mm responded with the lowest frequency of complete clinical closure. Nevertheless, complete furcation closure was achievable in 50% of molars with extensive bone loss. In agreement with that, Group III showed a more favorable outcome with the combined use of a vascularized MPM with DFDBA.

The enhanced improvement of the combined treatment group might be attributed to the unique action of MPM in bone healing (Malizos and Papatheodorou, 2005) when coupled with DFDBA that has a potential role in augmenting the healing process. It acts as a space-maker for the blood clot and prevents barrier collapse in the osseous lesion, allowing osteoconduction for new bone formation (Zenobio and Shibli, 2004).

Additionally, numerous animal experiments indicated that demineralization and freeze-drying of cortical bone allograft greatly enhanced its osteogenic potential. However, it was noted that the allograft can provide only initial mechanical support of the regenerative process. Preparation of a bone graft by freezing or radiation largely destroys its vital cellular components, reducing its osteogenic capacity (Van der Donk *et al*., 2003).

To maintain good long-term PR, adequate new bone formation is needed. Periosteum plays a major role in promoting bone growth and repair, and contains multiple cells with osteoblastic potential through the maintained proliferative capability of the transplanted cambium layer (Barckman *et al*., 2013). In particular, periosteum plays a unique role in revascularization of the bone graft in the early stages of healing (Yang *et al*., 2014).

Given that the healing process is a highly orchestrated and structured process, gingival wound healing is important to the periodontal surgery outcome (Kim *et al*., 2011). Therefore, it was very beneficial to examine the ultra-structural events associated with the early gingival wound healing in each line of treatment. Although 10 days may be too short a duration to observe structural changes, other authors found a considerable difference in both the cellular and extracellular phases of grafted and non-grafted sites in the same time frame (Lafzi *et al*., 2007).

Fortunately, our TEM results were in harmony with the clinical findings. Concerning the epithelial phase, the results showed obvious improvement in the two test groups. In view of the connective tissue phase, non-classical apoptotic features were revealed consistent with OFD sites in terms of cytoplasmic vacuolization, chromatin condensation and swollen mitochondria. The ECM mainly contained sparse, fragmented, incompletely formed collagen fibers and shrunken fibroblasts, indicating that OFD is accompanied by para-apoptotic changes that interfere with PR (Cattaneo *et al*., 2003).

On the other hand, Group II showed numerous proliferating fibroblasts and abundant collagen bundles. From the biological point of view, a protective effect of MPM may induce earlier healing features by enhancing proliferation and differentiation of the resident progenitor cells and those supplied by the cambium layer (Lin *et al.,* 2013). Interestingly, a new look should be taken towards an immune response coming from the periosteum, as

major histocompatibility complex II positive immune cells were detected in the cambium, suggesting the presence of dendritic cells (Frey *et al*., 2013).

In Group III, active fibroblasts and well organized collagen fibers were reflected; suggesting that the combination therapy favored the transformation of fibroblasts to metabolically active (formative) cells, which in turn enhance colonization of the root surface. Similarly, a recent histological study reported that the bioactivity of morselized allograft bone was found to be highlighted when added to the transplanted cambium owing to its content of bone-competent cells (Barckman *et al*., 2013). Moreover, it was suggested that periosteum may prevent soft tissue infiltration into the bone graft, and can inhibit osteoclasts that originate from the recipient site, reducing bone graft resorption compared to block graft alone (Yang *et al*., 2014).

Overall, the semilunar approach is proposed to give better results than the previous techniques described in the literature owing to the dual blood supply; firstly from the pedicle periosteum and secondly from the periosteum present below the furcation. In that way, the osteoinductive mechanism of MPM may be multi-factorial; the cambium layer maintains its relation to the original periosteum tissue, which might extend its regenerative effect on avascular roots. In addition, the potential liberation of essential bone-stimulating substances acting immediately once the cambium cells touch it may provide a signal for improved regenerative response, facilitating initial bone healing with better predictability.

The limitation of this technique remains that it cannot be used for cases with thin gingival biotypes. Also, long-term results are yet to be assessed. However, healing of periosteal grafts and good achievable results makes it a viable procedure for class II furcation defects.

Conclusion

In light of the findings of this study, it was demonstrated that the semilunar approach used in harvesting MPM represents a promising way to get a better clinical situation with a more predictable amount of PR. It represents also an alternative explanation for the potential efficacy of MPM in gingival wound healing. Meaningful improvements in both clinical parameters and features of gingival wound healing were revealed with the combination of MPM and DFDBA, supporting their adjunctive use in treatment of class II furcation defects.

Recommendation

Larger randomized clinical trials are needed to fully evaluate whether combined graft and MPM procedures offer an advantage over MPM alone. Additionally, longitudinal studies with long-term follow-up are needed to better quantify the value of such regenerative and economic techniques in improving the survival rate of furcation-affected molar teeth.

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