# Clinical Re-entry and Histologic Evaluation of Periodontal Intrabony Defects Following the Use of Marginal Periosteal Pedicle Graft as an Autogenous Guided Tissue Membrane

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**Abstract**

**Objective:** The aim of the present study was to clinically and histologically examine the effects of a marginal periosteal pedicle graft as a biologic guided tissue membrane**. Material and methods:** Fifteen patients with severe chronic periodontitis participated in this prospective, controlled, blind trial. Each paired periodontal defect was clinically evaluated for nine months. Nine months following therapy at re-entry, samples of the newly formed tissue were harvested from all test and control sites. Hopeless teeth of test and control samples were extracted for histologic evaluation of root surfaces exposed to the pocket environment. **Results:** At nine months, the experimental group showed a significant improvement in pocket depth reduction, clinical attachment level gain and intrabony defect reduction when compared to the open flap debridement control ($p < 0.01$). Histologic evaluation of test samples revealed coarse-fibered woven bone filling the defect nine months following therapy in three of the 10 examined newly formed tissue samples. Apical root notches of such samples showed a homogenous layer of cementum-like tissue deposition. **Conclusion:** Placement of a vascularized marginal periosteal pedicle graft as a barrier membrane significantly improved clinical and histologic parameters of deep periodontal intrabony defects.

*Key words: Guided tissue membrane, intrabony defects, chronic periodontitis, periodontal regeneration, periodontal flaps, clinical trials, histologic evaluation*

## Introduction

The aims of any periodontal therapy are to arrest and control periodontal infection and ultimately to regenerate lost periodontal structures. Because of differences in the healing abilities of different periodontal tissues, full regeneration of the periodontium following different periodontal treatment modalities has been difficult to achieve (Cho *et al.,* 1995). One of the most important factors that limit the achievement of a predictable regeneration is downgrowth of the junctional epithelium along the denuded root surface (Egelberg, 1987; Wirhlin, 1981).

A number of resorbable and non-resorbable guided tissue regeneration (GTR) materials have been proposed to delay epithelium downgrowth during healing and to provide an opportunity for the progenitor cells of the periodontal ligament and bone to repopulate previously diseased root surfaces. Several studies in animals (Nyman *et al.,* 1982; Caffesse *et al.,* 1988) and in humans (Gottlow *et al.,* 1986) have demonstrated that different levels of periodontal regeneration can be achieved with GTR. Non-resorbable membranes made of expanded polytetrafluorethylene (ePTFE) are the most widely investigated barrier membrane (Caffesse *et al.,* 1990; Pontoriero *et al.,* 1992; Walters *et al.,* 2003). The use of bioresorbable barriers such as collagen, polylactic acid, polyglycolic acid (Greenstein and Caton, 1993) was found to solve several of the shortcomings of the non-resorbable membranes. Bioresorbable barriers eliminate the need for a second surgery to remove the membrane and decrease the associated disturbances of the newly formed osteoid that may result in bone resorption.

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Multiple factors influence the predictability of treatment outcomes following GTR procedures. Clinically, the amount of new attachment achieved may be directly related to patient factors (oral hygiene, smoking and systemic health), root anatomy and the surgical technique (Gottlow *et al.,* 1986; Becker and Becker, 1990; Lu, 1992; Cortellini *et al.,* 1994). The degree of periodontal destruction and the remaining periodontal-osseous components (depth, width and number of osseous walls) are other critical factors that may cause wide variations in the outcome of the guided tissue regeneration. These factors can limit the number of progenitor cells and the amount of biologic mediators required to repopulate periodontal defects.

The periosteum, a structure rich in osteoprogenitor cells, has been viewed as having regenerative potential (Ishida *et al.,* 1996; Ueno *et al.,* 2001). Periosteal grafts could have the potential to stimulate bone growth because they stimulate osteogenic factors. Periosteal grafts also provide the wound area with additional osteoprogenitor cells, which may compensate for the deficiency of cells available in the periodontal defect. In addition, the periosteum can provide a rigid enough barrier to maintain the space of the periodontal defect to allow cells to migrate in and regenerate lost periodontal tissues. Polimeni *et al.* (2005) reported that space provision has a significant effect on alveolar bone regeneration in periodontal sites. The physiologic mode of growth factor delivery that could be achieved with periosteal osteoprogenitor cells could offer a great advantage of using the periosteum in periodontal regenerative therapy (Yu *et al.,* 1997, Gamal *et al.,* 1998, Gamal and Mailhot, 2000, Marzouk *et al.,* 2007).

Tobón-Arroyave *et al.* (2004) described in two case reports the usefulness of free periosteal grafts as barriers for bone regeneration in peri-radicular surgery when advanced periodontal breakdown occurs. They reported that the use of periosteal grafts in surgical therapy of combined periapical–periodontal lesions might contribute to a successful clinical outcome. Lekovic *et al.* (1991) and Kwan *et al.* (1998) clinically analyzed the efficacy of a palatal donor connective tissue graft including the periosteum as a barrier to enhance osseous regeneration. They reported a significant reduction in pocket depth, gain in attachment level, and inter-radicular bone fill in the experimental group when compared to that of the open flap debridement control. Hirokazu *et al.* (2006) described a novel approach to regenerate canine periodontal tissue defects by grafting autologous cultured membrane derived from the periosteum. They reported that in cases of cultured periosteum, the bone defects were regenerated and filled with newly formed hard tissue.

Gamal and Mailhot (2008) introduced a novel marginal periosteal pedicle flap as a biologic guided tissue regeneration membrane in the management of deep angular two-and three-wall infrabony periodontal defects. They reported that the use of vascularized marginal periosteal pedicle (MPP) graft as a barrier membrane significantly improved clinical and radiographic parameters of deep intrabony defects and proved superior to open flap debridement (OFD) alone.

In an attempt to evaluate the type of healing following the use of vascularized periosteum as an autogenous guided tissue regeneration membrane, this study was designed to clinically and histologically evaluate the use of marginal periosteal pedicle flaps for treating interproximal intrabony periodontal defects and compare their outcomes with open flap debridement.

## Materials and methods

### *Study population and design*

Fifteen non-smoking patients (11 males and 4 females) who were 27 to 45 years of age at the time of baseline examination (mean age 34.9 ± 5.5) with severe chronic periodontitis participated in this prospective, controlled, blinded trial. Patients were selected from individuals seeking care for periodontal problems at the Department of Periodontology of the Faculty of Dental Medicine, Al Azhar University, Cairo, Egypt. The criteria implemented for patient inclusion were: 1) no systemic diseases which could influence the outcome of the therapy; 2) good compliance with the plaque control instructions following initial therapy; 3) teeth involved were all vital; 4) each subject contributed matched pairs of two- or three-walled intrabony interproximal defects in premolar or molar teeth; 5) selected pocket depth (PD) > 6 mm and clinical attachment level (CAL) > 4 mm three weeks following initial cause-related therapy; 6) the selected 2- or 3-wall intrabony defects depth ranged from 3-6 mm as detected in diagnostic periapical radiographs; 7) the facial surface of teeth adjacent to the interproximal defect were free of extensive recession and marginal bone loss with at least 4-5 mm band of keratinized gingiva to allow for periosteal manipulation; 8) availability for the follow-up and maintenance program. Five of the selected patients had contralateral teeth diagnosed as hopeless for periodontal reasons and designated for extraction. Pregnant females were excluded from participating in the study. Patients also were excluded from the study if they presented with opened interproximal contact, or inadequate compliance with the oral hygiene maintenance schedule. Research procedures were explained to all patients and they agreed to participate in the study and signed the appropriate informed consent form of Al Azhar University. The experimental protocol was approved by the Ethical Committee of El Azhar University.

Initial cause-related therapy consisted of a thorough full mouth scaling and root planing for all teeth, performed in quadrants under local anesthesia. This procedure was performed using a combination of hand and ultrasonic instrumentation. Hand instruments were sharpened whenever necessary. Patients were recalled every other day for three weeks and received detailed mechanical plaque control instructions, which consisted of brushing using a soft toothbrush with a roll technique, and flossing. Supra-gingival plaque removal was performed whenever necessary.

Occlusal stents were fabricated with cold-cured acrylic resin on a cast model obtained from an alginate impression. The occlusal stent was made to cover the occlusal surface of the tooth being treated and the occlusal surfaces of at least one tooth in the mesial and distal directions. Stents were also extended apically on the buccal and lingual surfaces so as to cover the coronal third of the teeth involved. A pencil mark was made where the probe made contact with the acrylic stent and a groove was made on the pencil-marked area with a cylindrical low-speed burr. Using the groove as guide, the periodontal probe was re-inserted into the pocket during evaluation and re-evaluation procedures. Moreover, in order to eliminate inter-examiner error, the same trained operator (MAE), who was not aware of the type of treatment rendered, always collected the clinical data.

Three weeks following initial cause-related therapy, a re-evaluation was performed to confirm the need for periodontal surgery. Criteria used to indicate that surgery was required included the persistence of two interproximal sites with PD > 6 mm, CAL > 4 mm and having interproximal intrabony defects of > 3 mm. For a patient to serve as his own control, the study used a split mouth design where two interproximal contralateral defects were randomly (toss of a coin) assigned to either the control or experimental group. Experimental sites underwent periosteal graft defect coverage while control sites were treated with open flap debridement. All surgeries were performed by the same operator (AYG).

Baseline data for the experimental and control defects were collected just prior to the surgical phase of treatment and included plaque index (PI, Silness and Löe, 1964), gingival index (GI, Löe, 1967), and bleeding on probing (BOP) by visual inspection (gingival bleeding within 15 seconds after probing). With the acrylic stent in position, PD and CAL were recorded to the nearest millimeter at the deepest location of the selected interproximal site. Pocket depth and CAL measurements were taken with a calibrated periodontal probe with William's markings as the distance from the bottom of the pocket to the most apical portion of the gingival margin and the cementoenamel junction (CEJ) respectively (*Figure 1*).

Routine diagnostic periapical views utilizing intraoral size 2 dental films (Kodak Extraspeed, Eastman Kodak, Rochester, NY) were recorded by using the long cone paralleling technique and holders that were guided in a standardized position with the aid of a heavy bodied rubber impression customized bite block (Rinn Centering Device, Dentsplay Ltd., Weybridge, U.K.), employing an X-ray unit (Heliodent 70, Siemens, Bensheim, Germany) operating at 70 kV, 10 mA and 0.8 second exposure time to estimate the depth of the intrabony component before and after surgery. All radiographic examinations were performed with the evaluator blinded to the treatment assignment and unaware of the defect morphology observed during the surgery. All radiographs were digitized using a CCD-video camera with a standard distance between the radiographs and the camera. Distances from the most coronal position of the alveolar bone crest to the most apical extension of the intrabony destruction were identified on the scanned radiographs.

### *Surgical procedure*

All operative procedures for experimental and control sites were performed under local anesthesia (Mepicaine L, Kepivacaine HCL 2%, 1.8 ml, The Alexandria Company for Pharmaceuticals, Alexandria, Egypt). Local anesthesia was achieved with regional nerve block and infiltration. The surgical procedure of marginal periosteal pedicle flaps for the experimental sites was performed as described in detail previously by Gamal and Mailhot (2008). Briefly, it consisted of facial and lingual intrasulcular incisions one tooth mesial or distal to the interproximal defect, preserving as much keratinized tissue as possible. The tooth that possessed wider attached gingiva was selected as the donor site. Subsequently, one facial vertical releasing incision extending into the alveolar mucosa was carried out to fully uncover the facial periosteum and access the donor graft tissue site. Facial split thickness flap reflection in a supra-periosteal fashion was raised to a level that would permit free movement of a 3-4 mm wide periosteal pedicle strip. A Bard-Barker #15 blade was used to sharply dissect the flap as close to the periosteum as possible in apico-coronal movements so as to provide an approximately 2-2.5 mm uniformly thick soft tissue flap wall. The flap was extended under tension as the incision extended apically. Care was exercised not to perforate the flap base accidentally with the scalpel and to preserve as much interproximal soft tissue as possible. Full thickness mucoperiosteal flaps were raised lingually to a depth of 2-3 mm to allow for good debridement and better adaptation of the periosteal pedicle.

Following partial thickness soft tissue flap elevation, a marginal periosteal strip was obtained from the facial periosteum adjacent to the defect using one vertical inci-

sion starting from the alveolar crest 4 mm apically and one horizontal incision parallel to the gingival margin. The periosteum was harvested with a periosteal elevator under tension as separation extended laterally, keeping its base attached for use as a pedicle biological barrier membrane (*Figure 2*).

Debridement of all inflammatory granulation tissue from the intrabony defect was performed until a sound, healthy bone surface was obtained (*Figure 3*). The teeth were root planed thoroughly using hand and ultrasonic instruments to obtain a smooth, hard root surface. Periodontally exposed parts of the root surfaces were then etched with pH neutral ethylenediaminetetraacetic acid (EDTA gel 24%, Straumann PrefGel, Basel, Switzerland.) for 3 minutes (Gamal and Mailhot, 2003) followed by adequate rinse with a stream of water from a 3-way syringe for 10 seconds to remove residual EDTA.

During surgery, the following measurements were performed; distance from the cementoenamel junction to the bottom of the defect (CEJ-BD), and distance from the CEJ to the alveolar crest (CEJ-AC), the intrabony component (IBC) was defined as (CEJ-BD)-(CEJ-AC). The clinical IBC measurements were compared with the measurements obtained to detect the IBC from radiographs in order to evaluate reproducibility of the follow-up radiographic measurements of bone fill. For teeth designated for extraction notches were placed at the most apical end of the defects using a small round bur (diameter 2 mm) to indicate the most apical extent of periodontal destruction and serve as an orientation point during histologic evaluation nine months following therapy.

Periosteal flap material was rotated to cover the interproximal defect without suturing since the adherence of the flap to the bony surfaces was sufficient (*Figure 4*). Finally, the soft tissue flap free tension was repositioned following removal of any remaining pocket lining or tissue tags to cover the entire periosteal graft. It was secured to the original position with interproximal and sling sutures using black silk (3-0) so as to be easily distinguished by the patient during brushing. No periodontal dressing was applied in any case. In a separate visit, the selected control sites underwent an open flap debridement that consisted of elevation of full-thickness mucoperiosteal flaps with thorough defect and root surface debridement. Clinical IBC measurements were also recorded during surgery. EDTA gel etching of the periodontally exposed areas of the root surfaces was performed before flap closure.

All patients received oral and written post-operative instructions. Patients were prescribed amoxicillin 500 mg, TID for one week. During this initial healing phase, plaque control efforts were supplemented with chlorhexidine mouthrinse for one minute (0.12% chlorhexidine digluconate, Oraldine, Egypt) three times daily for the first post-operative month. Meanwhile, patients refrained from toothbrushing and interdental cleaning at the surgical areas.

Sutures were removed 14 days post-operatively and recall appointments for observation of any adverse tissue reaction and oral hygiene reinforcement were scheduled every second week during the first two months following surgery, then once a month during the rest of the observation period. All patients were instructed to resume their normal mechanical oral hygiene measures, which consisted of brushing using a soft toothbrush with a roll technique, and flossing, two weeks following surgery. Supportive periodontal maintenance of oral hygiene reinforcement and supragingival scaling, whenever necessary, were performed during each recall appointment.

Clinical and radiographic measurements were reassessed at three, six and nine months after surgery in order to evaluate the quantitative changes in the defect and to do critical comparisons with the preoperative films. Surgical re-entries were performed nine months after the initial therapy. The surgical re-entries consisted of a conservative buccal full thickness mucoperiosteal flap exposing only 1-2 mm of alveolar bone (*Figure 5*). All measurements performed at the time of surgical procedures were repeated during re-entries. For histologic evaluation of the nature of the regenerating tissues, the most coronal part of the newly formed tissues was carefully harvested with the use of a small periodontal chisel, thus a minimum amount of mechanical trauma was applied. A small periodontal chisel was used to make two vertical cuts in the newly formed tissues to a depth of about 1-2 mm along the mesial and distal aspects. Another horizontal cut was performed to separate the specimen area. Five teeth in each group that were diagnosed as hopeless and designated for extraction were extracted nine months following therapy for histologic evaluation of their root surfaces coronal to the apical notch.

### *Sample processing*

For future identification of the examined root surfaces of extracted teeth, a pencil mark was placed on the interproximal surfaces facing the periodontally exposed root defects. Teeth and tissue samples were immediately placed for one week in jars containing 10% neutral formalin, labeled by tooth number and patient data. Samples were decalcified using EDTA (125 g/L) and sodium hydroxide as a buffer for three weeks. Washing with running water to remove any excess of the decalcifying agent was performed. This step was followed by dehydration in ascending grades of ethyl alcohol starting with 70% up to 100%, and methyl benzoate for one day followed by paraffin benzol for two hours to remove the alcohol residue. Samples were then bathed in three

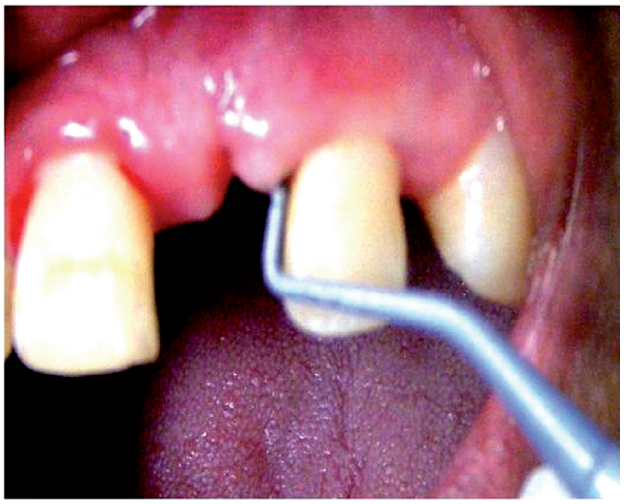

*Figure 1. Ten mm probe penetration affecting the mesial surface of the left upper lateral incisor.*

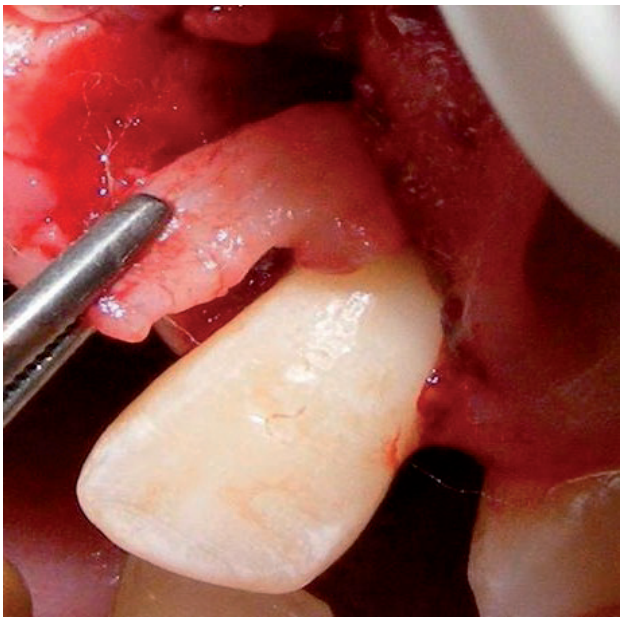

*Figure 2. Periosteal strip harvested and ready to cover the defect.*

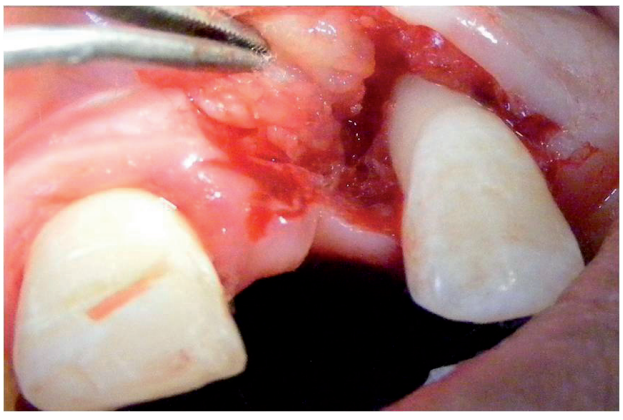

*Figure 3. Attachment loss (7 mm) was measured following defect debridement and root surface preparation.*

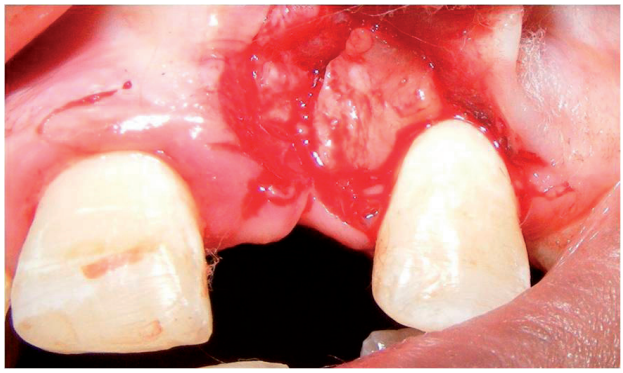

*Figure 4. Periosteal strip in position, completely covering the defect.*

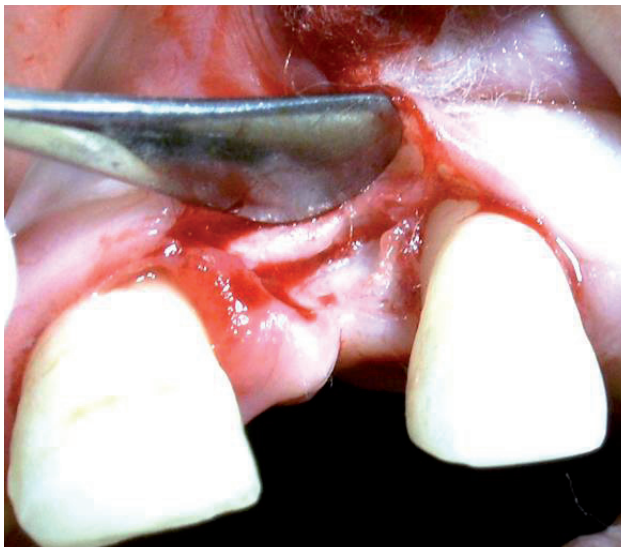

*Figure 5. Two mm attachment loss measured during re-entry surgery.*

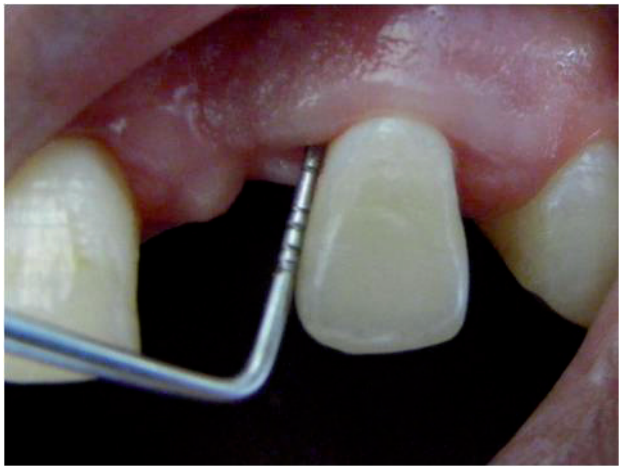

*Figure 6. Two mm probe penetration six months following marginal periosteal pedicle (MPP) graft therapy.*

changes of paraffin wax and placed in wax blocks of a size suitable for cutting. Transverse serial sections were cut from different levels of tissue samples in order to observe the whole circumference and depth. Tooth sectioning was performed in a mesio-distal direction in order to examine different deposited tissues in and around the apical notch. Cutting of the samples was done using a Leitz Wetzlar microtome making serial sections 4-6 μm thick. The sections were then stained with hematoxylin-eosin (H&E) and Mallory's stains (trichrome stain) for descriptive histological analysis by a blinded investigator (MGA).

## Data analysis

Statistical analysis of this study was carried out using SPSS – Release 12 for Windows. Numerical (quantitative) data are presented as mean ± standard deviation and categorical (qualitative) data are presented as frequencies and percentages (%). Initial differences between experimental and control groups were tested by paired *t*-test. It was also used to study the effect of time on numerical data of mean differences between the pre- and post-treatment values of each parameter. McNemar's Chi-square test was used to compare between categorical data of the experimental and control groups at each follow-up time period. Statistical significance was achieved when $p \leq 0.05$.

## Results

During the course of the study all patients showed good compliance and had uneventful post-operative healing in all of the experimental and control sites. All patients

**Table 1.** Baseline defect characteristics expressed in mm for both experimental (MPP) and control (OFD) groups.

| | MPP Mean ± SD | OFD Mean ± SD | *p* value |
|---|---|---|---|
| PD | 6.4 ± 1.4 | 5.8 ± 1.3 | 0.082 |
| CAL | 4.9 ± 1.1 | 4.8 ± 0.4 | 0.774 |
| IBC | 4.3 ± 0.7 | 4.5 ± 0.8 | 0.433 |
| PI | 0.9 ± 0.5 | 0.7 ± 0.5 | 0.189 |
| GI | 1.7 ± 0.5 | 1.7± 0.6 | 1.0 |
| BOP | 66.7% | 53.3% | 0.581 |

BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; IBC, intrabony component; MPP, marginal periosteal pedicle; OFD, open flap debridement; PD, pocket depth; PI, plaque index, SD, standard deviation.

**Table 2.** Comparison between baseline clinical and radiographic bone measurements in both groups.

| | MPP Mean ± SD | OFD Mean ± SD |
|---|---|---|
| Clinical | 4.3 ± 0.7 | 4.5 ± 0.8 |
| Radiographic | 4.5 ± 0.5 | 4.3 ± 0.6 |
| *p value* | 0.433 | 0.189 |

MPP, marginal periosteal pedicle; OFD, open flap debridement; SD, standard deviation

**Table 3.** Mean difference and degree of significance of the selected parameters in both experimental (marginal periosteal pedicle graft, MPP) and control (open flap debridement, OFD) groups compared to baseline during different observation periods.

| | | Pre-operative to 3 months | | Pre-operative to 6 months | | Pre-operative to 9 months | |
|---|---|---|---|---|---|---|---|
| | | Mean difference | *p* value | Mean difference | *p* value | Mean difference | *p* value |
| PD | Experimental | 3.2* | <0.001 | 3.6* | <0.001 | 3.8* | <0.001 |
| | Control | 2.1* | <0.001 | 2.5* | <0.001 | 2.5* | <0.001 |
| CAL | Experimental | 3.2* | <0.001 | 3.2* | <0.001 | 3.4* | <0.001 |
| | Control | 1.9* | 0.003 | 2.3* | 0.003* | 2* | <0.001 |
| IBC | Experimental | 2.4* | <0.001 | 2.5* | <0.001 | 3.2* | <0.001 |
| | Control | 1.6* | 0.014 | 1.6* | 0.023 | 1.6* | <0.001 |
| PI | Experimental | 0.3 | 0.164 | 0.3 | 0.164 | 0.3 | 0.055 |
| | Control | 0.2 | 0.082 | 0.2 | 0.334 | 0 | 0.103 |
| GI | Experimental | 0.8* | 0.013 | 0.9* | 0.004 | 0.7* | 0.016 |
| | Control | 1.1* | <0.001 | 1.1* | <0.001 | 0.9* | <0.001 |
| BOP | Experimental | 33.3* | 0.180 | 40% | 0.109 | 40% | 0.109 |
| | Control | 20% | 0.453 | 6.7% | 0.107 | 33.3% | 0.063 |

*Statistically significant difference. BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; IBC, intrabony component ; PD, pocket depth; PI, plaque index

**Table 4.** Mean defect characteristics of both experimental (marginal periosteal pedicle graft, MPP) and control (open flap debridement, OFD) groups expressed in mm ± SD during different observation periods.

| | PD | | | CAL | | | IBC | | | PI | | | GI | | | BOP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3M | 6M | 9M | 3M | 6M | 9M | 3M | 6M | 9M | 3M | 6M | 9M | 3M | 6M | 9M | 3M | 6M | 9M |
| MPP | 3.2 ± 0.8 | 2.7* ± 0.6 | 2.6* ± 0.6 | 1.7* ± 0.6 | 1.7* ± 0.6 | 1.5* ± 0.5 | 1.9* ± 0.6 | 1.9* ± 0.5 | 1.1* ± 0.5 | 0.6 ± 0.6 | 0.6 ± 0.6 | 0.5 ± 0.7 | 0.9 ± 0.7 | 0.7 ± 0.8 | 0.9 ± 0.7 | 33.3% | 26.7% | 26. 7% |
| OFD | 4.2 ± 0.8 | 4.1 ± 0.7 | 4.1 ± 0.8 | 2.9 ± 0.9 | 2.5 ± 0.9 | 2.8 ± 0.8 | 2.9 ± 0.7 | 2.9 ± 0.5 | 2.9 ± 0.6 | 0.9 ± 0.6 | 0.9 ± 0.7 | 0.7 ± 0.5 | 0.6 ± 0.6 | 0.5 ± 0.5 | 0.7 ± 0.6 | 33.3% | 46.7% | 20% |
| *p* value | 0.089 | 0.027 | 0.016 | <0.001 | 0.003 | 0.001 | 0.005 | 0.001 | 0.001 | 0.301 | 0.389 | 0.546 | 0.334 | 0.384 | 0.384 | 0.302 | 0.481 | 0.057 |

*Statistically significant difference. BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; IBC, intrabony component; PD, pocket depth; PI, plaque index, SD, standard deviation.

**Table 5.** Percentage of intrabony component (IBC) fill and clinical attachment level (CAL) measurements (mm) nine months following therapy in both groups:

| | MPP (n = 15) | OFD (n = 15) |
|---|---|---|
| IBC | | |
| 0 | 0.15 | 0 |
| 1 | 73.3 | 0 |
| 2 | 20 | 26.6 |
| 3 | 0 | 60 |
| < 3 | 0 | 13.3 |
| CAL | | |
| 0 | 0 | 0 |
| 1 | 53.3 | 0 |
| 2 | 46.6 | 40 |
| 3 | 0 | 40 |
| < 3 | 0 | 20 |

tolerated the surgical procedures well; no any site had to be eliminated from the study and no cases of flap dehiscence or infection were detected. Minimal swelling of the soft tissues surrounding the operated areas was observed during the early days of healing. With regard to the number of bony walls of treated defects, distribution was as follows: of MPP sites, eight were predominately 2-wall and seven were predominately 3-wall, and of OFD sites, nine were predominately 2-wall and six were predominately 3-wall.

A summary of baseline defect characteristics three weeks following completion of cause-related therapy for both groups is provided in *Table 1*. No statistically significant differences were found pre-operatively between MPP and OFD groups with respect to soft and hard tissue measurements ($p < 0.05$). The defects had deep PDs (MPP, 6.4 ± 1.4 mm; OFD, 5.8 ± 1.3 mm) and were associated with deep intrabony defects (MPP, 4.3 ± 0.7 mm; OFD, 4.5 ± 0.8 mm). Similarly, CAL was 4.9 ± 1.1 mm and 4.8 ± 0.4 mm at experimental and control sites, respectively. In addition, no significant differences were found between the mean values of the clinical IBC obtained during surgery and the radiographic IBC obtained immediately after surgery (*Table 2*).

The mean difference and degree of significance of the selected parameters in both MPP and OFD groups were compared to baseline during different observation periods and are presented in *Table 3*. Both experimental and control groups showed significant reduction in pocket depth, gain in clinical attachment level and reduction in IBC depth when compared to baseline at the three follow-up observation periods ($p < 0.001$). By the end of the study, the mean differences in pocket reduction were 3.8 mm and 2.5 mm for the MPP and OFD groups, respectively (*Figure 6*). The mean differ-

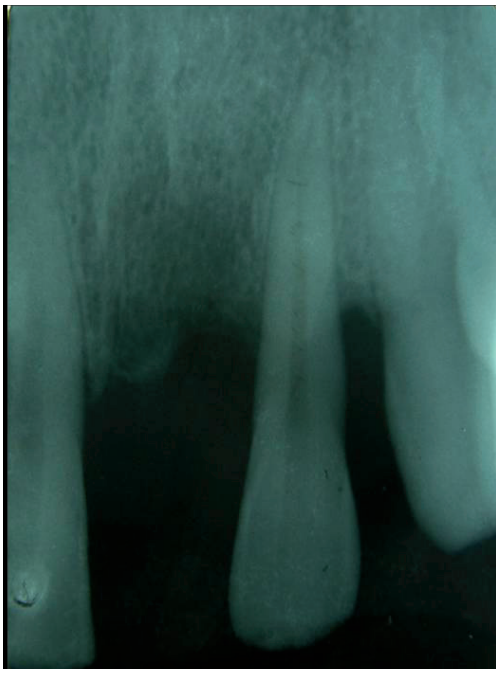

***Figure 7. Pre-operative view showing advanced bone destruction with vertical bone loss affecting the mesial surface and cup-shaped resorption affecting the distal surface.***

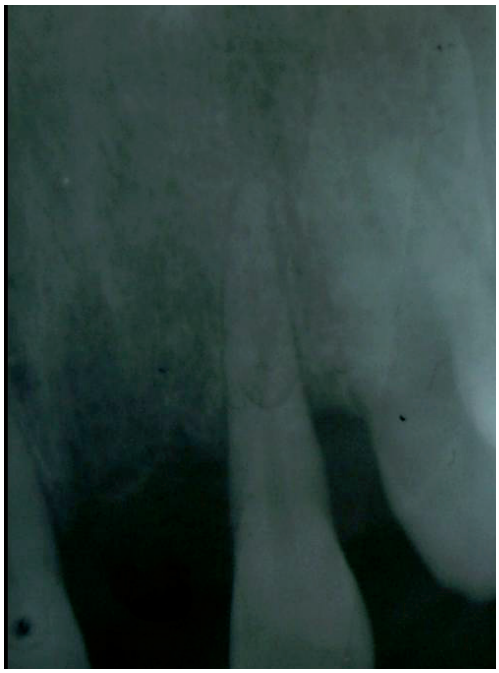

***Figure 8. Three-month post-operative periapical view showing bone fill on either side of the defect.***

ences in CAL were 3.4 mm for the experimental group and 2.0 mm. for the controls. The differences in IBC values were 3.2 mm and 1.6 mm. for MPP and OFD groups, respectively (*Figures 7,8*). Moreover, no significant alterations were documented in plaque scores or bleeding on probing in either group. The gingival index was significantly improved in both groups compared to baseline during all three observation periods.

*Table 4* summarizes the differences between the MPP and OFD groups at the three clinical observation periods. Gingival index, plaque index and percentage of BOP remained low throughout the study, irrespective of the treatment modality. Mean pocket depth at nine months was 2.6 ± 0.6 mm for the MPP group and 4.1 ± 0.8 mm for the control group, presenting a significant difference favoring the MPP group ($p < 0.05$). The magnitude of the observed additional benefit by the end of the study was 1.5 ± 0.7 mm.

The mean CAL by the end of the study was 1.5 ± 0.5 mm for the MPP group, while the OFD group had a mean of 2.8 ± 0.8 mm. The differences were significantly greater for the experimental group when compared to that of the control group ($p = 0.001$). The magnitude of the observed additional benefit at nine months was 1.3 ± 0.5 mm. At nine months, the percentage of sites that showed CAL gain of 0-1 was 53.3 for the MPP group compared to 0 for the OFD group (*Table 5*).

As shown in *Table 5*, the MPP group yielded statistically significant improvements in bone fill compared to the OFD group nine months following surgery ($p < 0.001$). The mean IBC level measured during surgical reentries at the nine month observation period was 1.1 ± 0.5 mm for the MPP experimental group and 2.9 ± 0.6 mm for the OFD control. The magnitude of the observed additional benefit at nine months was 1.8 ± 0.5 mm. The mean defect fill compared to baseline was statistically higher in MPP graft-treated defects compared to OFD-treated defects (3.2 mm and 1.6 mm, respectively). By the end of the study, the percentage of sites that showed IBC of 0-1 was 73.18 for the MPP group compared to 0 for the control (*Table 5*).

In three of the 10 examined root samples from the MPP group, histological analysis demonstrated a fibroblast-rich connective tissue layer extending for variable distances coronally, as measured from the bottom of the previous defect (3.4 ± 1.2 mm) (*Figures 9, 10*). Complete union between fibrous tissues apical and coronal to the notch was evident (*Figure 11*). Apical root notches of such samples showed a homogenous layer of cementum-like tissue deposition (*Figure 12*). Five of the test samples showed no cemental-like tissue deposition within the notch in spite of defect bony fill; some areas of root resorption were suggestive of bony ankylosis (*Figure 13*). In two samples from the MPP group, apical root notches could be identified, which hindered identification of the newly formed tissue and its subsequent evaluation.

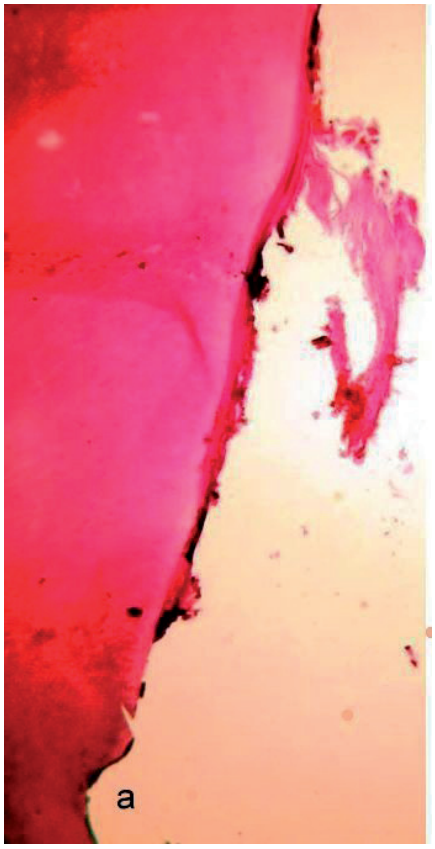


*Figure 9. Homogenous layer of cementum-like tissue deposition (darkly stained margin) including the apical notch area (a) over experimental root sample nine months following therapy. Original magnification x 400, H&E.*

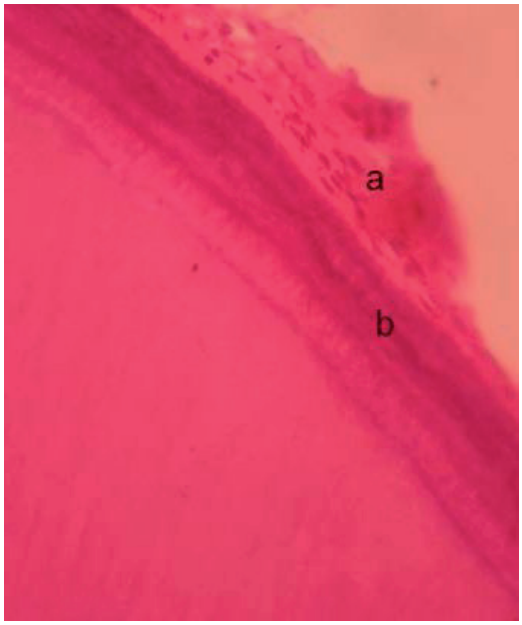


*Figure 10. Closer view of an area coronal to the apical notch showing new fibroblasts, rich connective tissue attachment (a) and cementum-like tissue deposition (b). Original magnification x 800, H&E.*

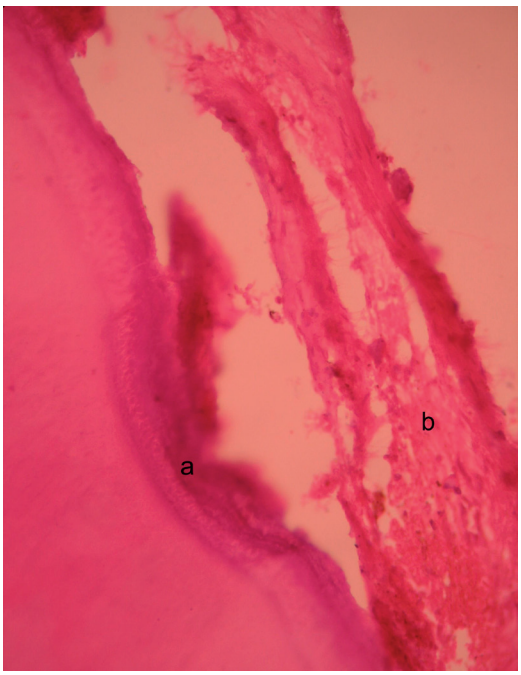


*Figure 11. Experimental root sample showing complete union between newly formed fibrous tissues coronal to the notch and old fibrous tissue apical to the notch. Original magnification x 400, Mallory's stain.*

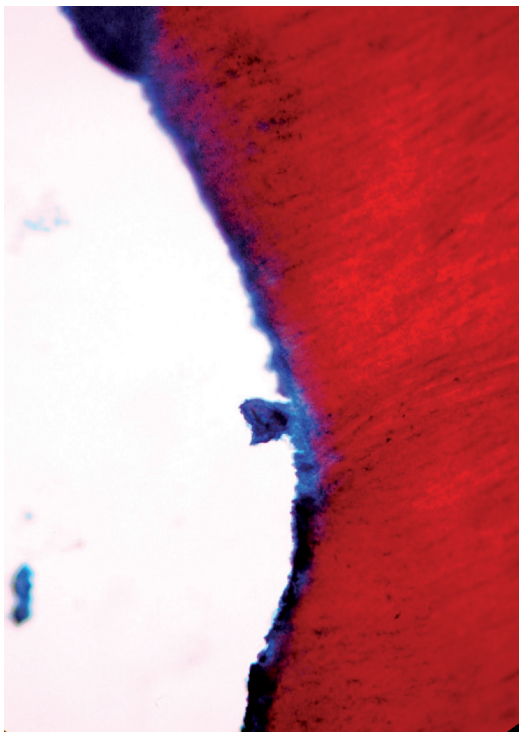

*Figure 12. Apical notch of a test sample showing homogenous layer of cementum-like tissue deposition (darkly stained notch border). Original magnification x 400, Mallory's stain.*

Control root samples were covered by a layer of granulation tissue, with no evidence of cementum-like tissue deposition or fibrous attachment within the apical notch (*Figure 14*). Two cases showed interrupted cementum-like deposition limited to the most apical part of the notch area with no evidence of fibrous tissue deposition. Microscopic analysis of tissues harvested from the most coronal part of the newly formed tissues of all samples treated by periosteal defect coverage revealed new bone formation that was distinct from mature cortical bone. The newly formed bone lacked trabecular architecture and demonstrated variability in staining intensity. Mature lamellar bone was also observed surrounding immature bone (*Figure 15*). Two samples showed marginal fibrous tissue inserted into the newly formed bone, suggesting periodontal ligament attachment (*Figure 16*). Closure of the control defects appeared to have been essentially accomplished by soft tissue. Most of the tissues harvested from the control OFD-treated sites revealed inflammatory cell infiltrate of varying extent and red blood corpuscles entrapped in a fibrin meshwork. In addition, homogeneous degenerative tissues indicating necrosis and dense fibrous tissue formation were also evident. No osseous fill was observed in any area of the examined samples (*Figure 17*).

## Discussion

Regeneration of the periodontium is the result of elective cellular events that are facilitated by tissue exclusion using bioabsorbable or non-absorbable barriers. Several randomized clinical trials have indicated that resorbable and non-resorbable GTR materials lead to statistically significant increases in periodontal clinical attachment levels, but the magnitude of the observed additional benefit was modest (Trombelli *et al.*, 2002). Many other studies reported no clinically significant differences between the test resorbable and non-resorbable barrier membranes and open flap debridement control (Mellado, *et al.*, 1995; Wallace, *et al.*, 1994; Bratthall *et al.*, 1998). Following advanced periodontal destruction, the limited regenerative capacity of the remaining periodontal structures represents the most important factor that restricts predictability of the current GTR treatment outcome. The periosteum, as a structure rich in osteoprogenitor cells, has been viewed as having regenerative potential (Ishida *et al.*, 1996; Ueno *et al.*, 2001) and could be a good alternative to the currently available GTR materials.

The pedicle periosteal graft that was employed in the present study could have the potential to stimulate osteogenesis in the periodontally involved area because of its ability to stimulate osteogenic factors. At the same time, periosteal grafts might provide the wound area with additional osteoprogenitors cells that may compensate for the deficient number of progenitor cells available in the periodontal defect. The physiologic mode of growth and differentiation factor delivery that could be achieved by periosteal osteoprogenitor cells offers a great advantage to using vascularized periosteum as a biologic barrier. In terms of viability, a vascularized marginal graft harvested from the periosteum provides a more adequate vascular supply to the graft. The osteogenic capacity of vascularized periosteum is less affected by the environment of the recipient site than is free periosteum (Gamal and Mailhot, 2008; Isheda *et al.*, 1996).

Clinically, the autogenous marginal periosteal pedicle grafts used in the present study seem to be a good alternative for synthetic guided tissue regeneration membranes. Harvesting the periosteum from the marginal areas of the tooth adjacent to the defect eliminates the disadvantages of the need for second surgery to harvest remote donor tissue. The periosteal pedicle flap design employed in the present study is believed to provide complete coverage and subsequent protection and enhanced healing capacity of the intrabony defect. The use of a partial thickness flap and harvesting the marginal periosteum in the design of the MPP flap was done to minimize the possibility of creating gingival recession in the donor area. Selection of donor tissue from an area with adequate attached gingiva and a thick gingival biotype are other factors that helped to avoid recession. In addition, the periosteum appeared to be rigid enough to maintain the periodontal defect space to be later repopulated by the regenerating periodontal tissues.

Clinical and radiographic measurements were reassessed starting three months after surgery to check for earlier healing following the use of the periosteal graft. The results of this study confirm preliminary findings described by Gamal and Mailhot (2008), where clinical attachment gain and pocket resolution were achieved by marginal periosteal pedicle flap coverage of intrabony defects and proved superior to treatment with open flap debridement. Treatment of the intrabony defects with the MPP flap resulted in statistically significant reductions in PD and gains in CAL compared to open flap debridement alone. The absolute values of the observed added benefit in our study nine months post-operatively were $1.5 \pm 0.7$ mm for PD and $1.3 \pm 0.5$ mm for CAL, which is roughly mid-way between the average outcomes previously reported in many investigations utilizing a similar experimental design with different resorbable and non-resorbable membranes. Tonetti *et al.* (2004) reported $0.8 \pm 0.3$ mm added CAL gain following treatment of intrabony defects with collagen membrane and bovine bone replacement material compared to papilla preservation access flap alone. They reported that it is not possible to ascertain whether or not the effect would have been observed following the application of a single component of the combination therapy. For that reason,

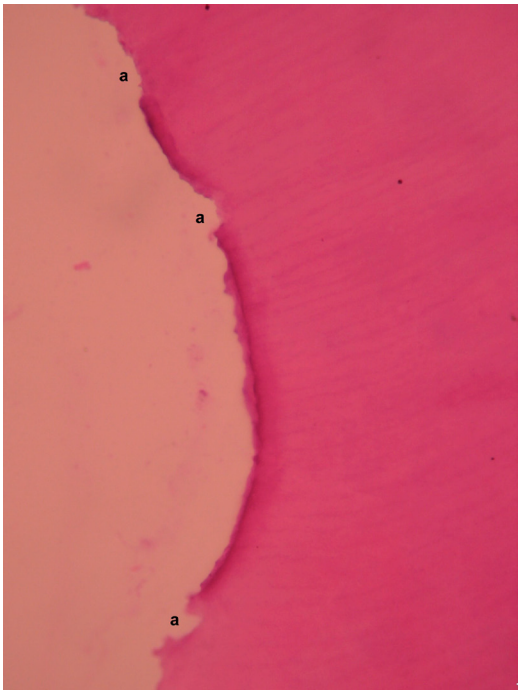


*Figure 13. Apical notch of a test sample showing interrupted cementum-like deposition (densely stained notch border). Areas of root resorption (a) interrupting the continuity of cementum-like tissues were evident. Original magnification x 400, H&E.*

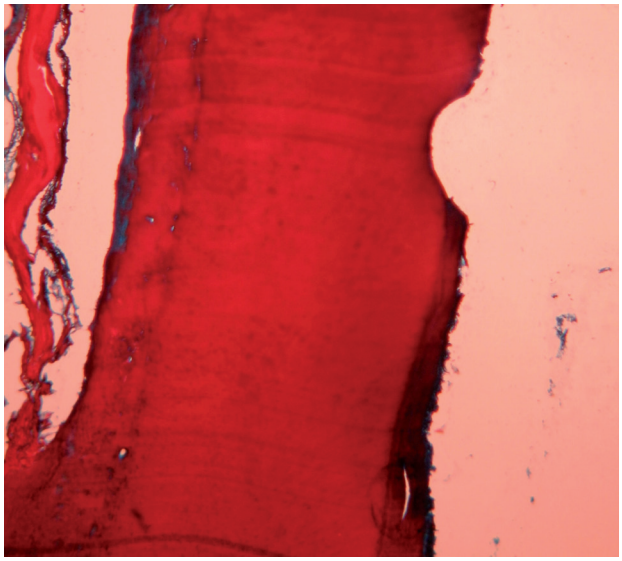

*Figure 14. Control root sample with no evidence of cementum-like tissue deposition or fibrous attachment within or coronal to the apical notch. Original magnification x 400, Mallory's stain.*

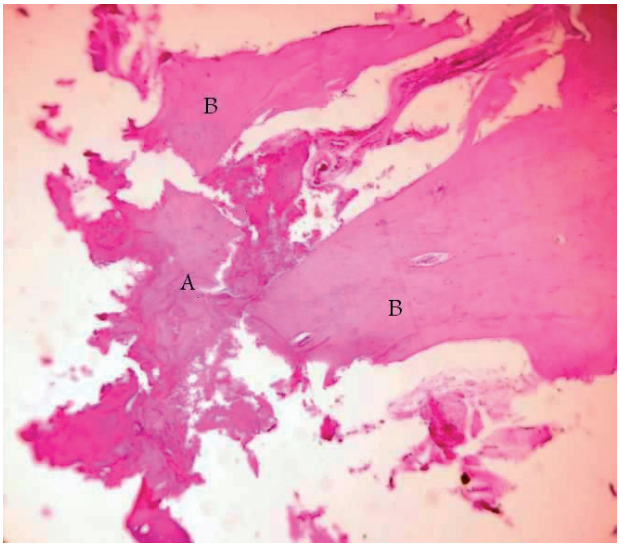


*Figure 15. Photomicrograph of test specimen nine months after therapy showing areas of immature woven bone (a) surrounded by areas containing more mature lamellar bone (b). Original magnification x 400, H&E.*

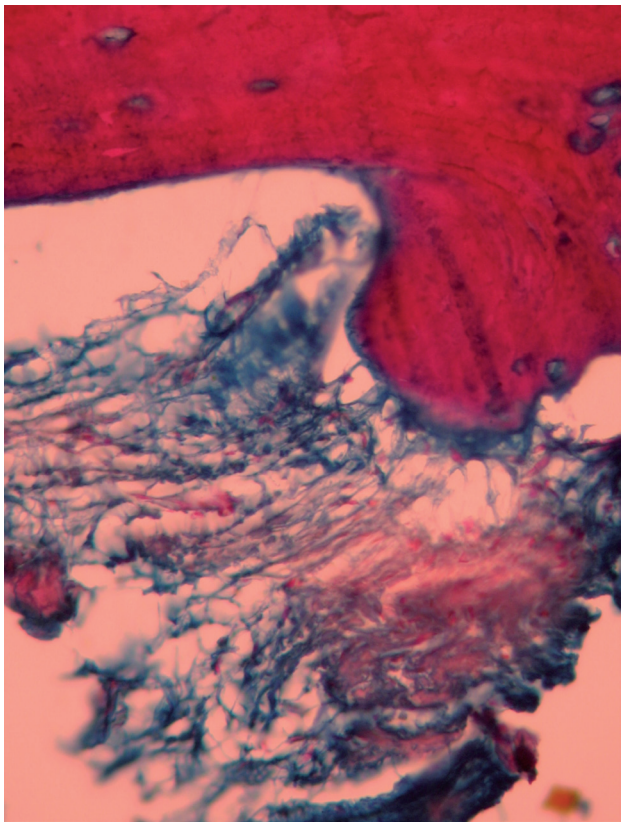

*Figure 16. Test sample showed marginal fibrous tissue inserted into the newly formed bone, suggesting periodontal ligament attachment. Original magnification x 400, Mallory's stain.*

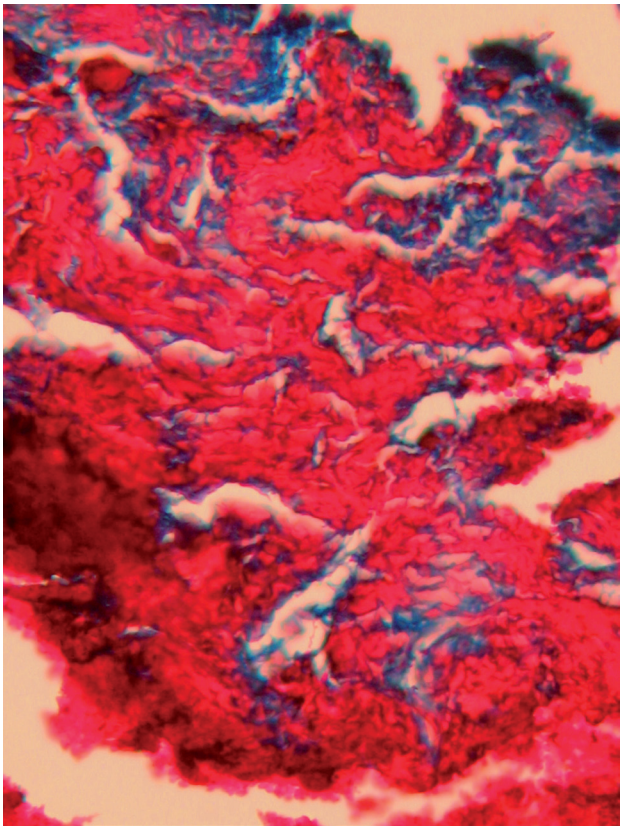

***Figure 17. Photomicrograph of tissues harvested from a control site nine months after therapy revealed granulation tissue formation (blue stain), inflammatory cell infiltrate, and red blood corpuscles entrapped in a fibrin meshwork (red stain). Original magnification x 400, Mallory's stain.***

the present study utilized the MPP flap without the application of any bone replacement therapy. Murphy and Gunsolley (2003) also reported a similar level of 0.95 ± 0.47 mm of added CAL benefit after application of collagen membrane to intrabony defects. Becker *et al.* (1987) and Gottlow *et al.* (1986) reported a mean gain in clinical attachment ranging from 0.56 mm to 4.1 mm following the use of non-resorbable membranes. Silvestri *et al.* (2003) reported attachment level gain of 4.3 ± 1.9 mm and pocket depth reduction of 5.6 ± 1.5 mm one year following ePTFE coverage. However, the mean baseline pocket depths and attachment levels of these studies were much higher than those of our present study (8.9 ± 1.9 mm and 8.1 ± 1.9 mm compared to 6.4 ± 1.4 mm and 4.9 ± 1.1 mm, respectively).

Bone fill data derived from surgical re-entry is important to substantiate routine post-operative measurement data as generated in this study. Nine months post-operatively, MPP experimental sites showed 3.2 mm intrabony defect depth reduction compared to 1.6 mm for the control sites. The difference in defect fill between two and three osseous walls is not included in the present study. The osteogenic potential of the covering vascularized periosteal barrier may explain the differences in defect fill observed between control and experimental groups. Lower levels of defect fill (1.18 mm to 1.26 mm.) have been reported by Lekovic *et al.* (2001) and Kwan *et al.* (1998) following the use of free palatal donor connective tissue as an autogenous guided tissue regeneration membrane. The amount of bone fill reported by Becker *et al.* (1987) and Gottlow *et al.* (1986) following the use of non-resorbable membranes was between 1.3 mm and 5.0 mm. Comparable bone defect improvements were observed in re-entry studies using porous hydroxyapatite either alone (Kenny *et al.*, 1988) or in combination with ePTFE (Lekovic *et al.*, 1991). Walters *et al.* (2003) reported 2.8 mm defect fill following the use of porous and non-porous ePTFE membranes.

The present study employed five teeth in each group that were assigned for extraction due to advanced periodontal breakdown, which in turn enabled histological documentation of the treatment results. In order to avoid violation of the ethical bases for patient evaluation, we did not resort to block sections in our histologic evaluation of the newly formed tissues. We decided to examine the newly formed tissues on either side of the periodontium separately. The root surface areas exposed to the pocket environment from one side were evaluated following tooth extraction, and the regenerating tissues were obtained during reentry surgery from the other side. In three of the 10 MPP root samples histological analysis demonstrated a fibroblast-rich connective tissue layer extending for variable distances coronally. Apical root notches of such samples showed a homogenous layer of cementum-like tissue deposition: such a healing pattern indicates that either the migration rate of periodontal ligament cells is at least as high as that of bone cells (Gottlow, 1986), or periosteal progenitors have differentiated into cementoblasts upon contact with the root surface. Five of the test samples had no cementum-like tissue deposition within the notch in spite of defect bony fill, and some areas of root resorption were suggestive of bony ankylosis. Gottlow (1986) reported that ankylosis should occur if granulation tissue derived from the bone makes contact with the root before granulation tissue originating from the periodontal ligament makes contact.

Several studies allowed the removal of a small amount of regenerating tissues without compromising further healing of the defect (Pritlove-Carson *et al.*, 1994; Liljenberg *et al.*, 1994; Berglundh *et al.*, 1999; Zitzmann *et al.*, 2005). In the present study, 1-2 mm tissue samples were harvested from the most coronal part of regenerating tissues. All test samples lacked trabecular architecture and demonstrated variability in staining intensity. From a biological point of view, the protective effect of the periosteal pedicle and the supposed physiologic delivery of growth and differentiation factors may induce earlier

bone growth by enhancing proliferation and differentiation of resident progenitor cells and those supplied by the periosteum.

Clinical re-entry and histologic data withdrawn from this study demonstrated that an MPP graft, an autogenous guided tissue membrane, will heal with a significant gain in attachment level and bone fill. It represents a promising way to get a more predictable amount of periodontal regeneration. Further investigations are required to explore the biological effects of such a modality on periodontal wound healing and to study its effects in more challenging situations (horizontal defects, one osseous wall defects, aggressive periodontitis, and smokers).

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