Gingival Crevicular Fluid Bone Morphogenetic Protein-2 Release Profile Following the Use of Modified Perforated Membrane Barriers in Localized Intrabony Defects: A Randomized Clinical Trial

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Abstract

Background: In guided tissue regenerative surgery, membrane perforations may serve as a mechanism for the passage of cells and biologic mediators from the periosteum and overlying gingival connective tissue into the periodontal defects. To test this assumption, this study was designed to evaluate levels of bone morphogenetic protein-2 (BMP-2) in gingival crevicular fluid (GCF) during the early stages of healing for sites treated with modified perforated membranes (MPMs) as compared with occlusive membranes (OMs).

Methods: Fifteen non-smoking patients with severe chronic periodontitis participated in this prospective, randomized and single-blinded clinical trial. Each patient contributed two interproximal contralateral defects that were randomly assigned to either an experimental modified perforated membrane group (15 sites) or a control occlusive membrane group (15 sites). Plaque index, gingival index, probing depth (PD), clinical attachment level (CAL) and the relative intrabony depth of the defect (rIBD) were measured at baseline and reassessed at three, six and nine months after therapy. Gingival crevicular fluid samples were collected on day 1 and 3, 7, 14, 21, and 30 days after therapy.

Results: The MPM-treated group showed a statistically significant improvement in PD reduction and clinical attachment gain compared to the OM control group. Similarly, rIBD was significantly reduced in MPM-treated sites as compared with those of the OM group. BMP-2 concentrations peaked in the MPM samples obtained during the early postoperative period (days 1, 3 and 7) with a statistically significant difference compared with OM-treated groups. BMP-2 levels decreased sharply in the samples obtained at days 14, 21 and 30 with non-significant higher levels in MPM samples as compared with those of OM sites.

Conclusion: Within the limits of the present study, one can conclude that MPM coverage of periodontal defects is associated with a significant initial increase in GCF levels of BMP-2, a factor that could improve the clinical outcomes of guided tissue regenerative surgery.

Key words: Periodontal regeneration, guided tissue membranes, bone morphogenetic protein, growth factors, periodontal pockets

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Introduction

Although guided tissue regenerative therapies have great potential, they remain unpredictable in their ability to consistently produce acceptable outcomes in all situations (Cho *et al.,* 1995). Perhaps the most important factor that would negatively affect guided tissue regeneration (GTR) is periosteal isolation. This barrier effect would deprive the wound area from the regenerative potential of the periosteum, including progenitor cells and biologic mediators. The periosteum has been shown to have significant regenerative potential (Ishida *et al.,* 1996). Periosteal grafts were found to have the potential to stimulate osteogenesis in periodontal defects by their capacity to upregulate osteogenic factors (Ueno *et al.,* 2001; Gamal and Mailhot, 2008). In addition, periosteal grafts were reported to contribute additional osteoprogenitor cells that would compensate for their relative deficiency in the defects (Gamal *et al.,* 2010; 2011).

The recent isolation of gingival mesenchymal stem cells (GMSCs) from gingival connective tissue has made it reasonable to reevaluate the protocol of gingival connective tissue isolation in GTR procedures. They have been shown to exhibit clonogenicity, self-renewal, and multipotent differentiation capacities (Mitrano *et al.,* 2010; Tomar *et al.,* 2010; Tang *et al.,* 2011). These cells are capable of immunomodulatory functions, specifically suppressing peripheral blood lymphocyte proliferation (Zhang *et al.,* 2009). At the functional level, mesenchymal stem cells (MSCs) display chemotactic properties similar to immune cells in response to tissue insult and inflammation, thus exhibiting tropism for the sites of injury via production of anti-inflammatory cytokines and anti-apoptotic molecules (Spaeth *et al.,* 2008; Karp *et al.,* 2009; Nauta and Fibbe, 2007). These unique characteristics of MSCs make them attractive candidates for the enhancement of periodontal tissue regeneration. Isolation of the wound area from this important source of GMSCs through the use of traditional occlusive guided tissue membranes may therefore limit the regenerative potential of GTR procedures.

Macroscopically, based on its larger surface area compared to that of the periodontal ligament, gingival connective tissue is highly vascular. In addition, gingival connective tissue represents the most abundant structural cell in periodontal tissue (Nanci and Bosshardt, 2006). Although many researchers suggested that gingival connective tissue cells lacked the potential for regeneration and occlusive GTR devices showed significantly greater bone regeneration (Polimeni *et al.,* 2004; Karring *et al.,* 1980; Nyman *et al.,* 1980), other experimental studies have reported that gingival connective tissue cells may contribute to the regenerative process (Aukhil and Iglhaut, 1988; Aukhil *et al.,* 1985; Aukhil *et al.,* 1986; Bowers and Donahue, 1988; Iglhaut *et al.,* 1988). *In vitro*, both gingival and periodontal ligament fibroblasts were found to express mRNA for BMP-2 and BMP-4 (Ivanovski *et al.,* 2001). Both cell types were also found to express hard tissue-associated proteins in osteogenic media and were able to synthesize and break down the collagen fibers and other proteins from the ground substance (Lallier *et al.,* 2005; Ivanovski *et al.,* 2001; Bartold and Narayanan, 2006).

Gamal and Iacono introduced a novel perforated collagen membrane as a modality that could enable participation of periosteal cells, gingival fibroblasts and gingival stem cells in GTR procedures (Gamal and Iacono, 2013). They demonstrated in a clinical study that the use of a modified perforated membrane (MPM) improved clinical outcomes significantly more than those observed with the use of occlusive membranes. The design of their study did not allow for the identification of which component(s) of the periodontium contributed to the positive results obtained. Clinical findings were not validated by further analysis to identify the nature of healing and whether gingival fibroblasts, GMSCs and/or periosteal cells contributed toward the enhanced regenerative process. It has also been suggested that growth and differentiation factors from cells in the periosteum and gingiva could pass through the membrane perforations and augment regeneration. Bone morphogenetic proteins (BMPs) are crucial differentiation factors in bone formation and healing (Reddi, 1998; Bessa *et al.,* 2008; Kanakaris and Giannoudis, 2008; Yu *et al.,* 2010; Reddi, 2005; Chen *et al.,* 2004). They possess very strong osteoinductive activity, induce differentiation of mesenchymal cells into chondrogenic and osteogenic cells, and promote osteoblast proliferation (Takiguchi *et al.,* 1999; King *et al.,* 1997; Jung *et al.,* 2003; Jepsen and Terheyden, 2002; Zhao *et al.,* 2003). In this study we studied levels of BMP-2, which is reported to be the most active member of the BMP phenotypes. A direct correlation could exist between the number of the available cells and their released growth and differentiation factors. To test this assumption, the objective of this study was to evaluate levels of bone morphogenetic protein-2 in GCF during the early stages of healing for sites treated with MPMs as compared with those sites treated with occlusive barrier membranes.

Materials and methods

Patient selection

Fifteen non-smoking patients (8 males and 7 females) who were 31 to 51 years of age at the time of baseline examination (mean age 33.8 ± 6.1 years) with severe chronic periodontitis (Armitage, 1999) participated in this prospective, split-mouth, randomized and single-blinded clinical trial. Since no previous data on GCF BMP-2 levels following the use of MPM or OM are available to provide data for sample size calculation, post-hoc power analysis was performed for the clinical

part of the study. The sample size was 7 subjects in each group at an alpha level of 0.05 (5%), and β level of 0.20 (20%). The obtained power was 81%. The subjects were recruited consecutively from the list of patients seeking periodontal treatment in the Department of Periodontology of the Faculty of Dental Medicine, Al Azhar University, Cairo, Egypt, between March 2012 and November 2012. 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 with no mobility; 4) each subject contributed matched pairs of 2- or 3-wall intrabony interproximal defects around premolar or molar teeth without furcation involvement; 5) selected 2- or 3-wall intrabony defects (IBD) measured from the alveolar crest to the defect base in diagnostic periapical radiographs of ≥ 4 mm; 6) selected probing depth (PD) ≥ 5 mm and clinical attachment loss (CAL) ≥ 4 mm at the site of intraosseous defects 4 weeks following initial cause-related therapy; 7) availability for the follow-up and maintenance program; 8) absence of periodontal treatment during the previous year; 9) absence of systemic medications that could affect healing or antibiotic treatment during the previous 6 months; 10) absence of a smoking habit; and 11) absence of occlusal interferences, mobility, open interproximal contacts (diastema, flaring or both). Pregnant females were excluded from participating in the study. Patients were also excluded from the study if they demonstrated inadequate compliance with the oral hygiene maintenance schedule. The experimental protocol was approved by the Ethical Committee of Al Azhar University (OMD - 45 – 2012). Research procedures were explained to all patients and they agreed to participate in the study and signed the appropriate informed consent form. This clinical trial was registered under a clinical trial registration number: NCT01860495.

Presurgical therapy and grouping

Initial cause-related therapy consisted of thorough full mouth scaling and root planing performed in quadrants under local anesthesia. This procedure was performed using a combination of hand and ultrasonic instrumentation using a P10 tip. Patients were recalled every 3 days for three weeks and received detailed mechanical plaque control instructions that consisted of brushing with a soft toothbrush in a roll technique and flossing. Supra-gingival plaque removal was performed whenever necessary. Four weeks after initial therapy, a reevaluation 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 ≥ 5 mm, CAL ≥ 4 mm, and interproximal intrabony component of ≥ 4 mm. Baseline periodontal

disease status of the selected sites was determined by clinical periodontal assessments, including plaque index (PI; Silness and Loe, 1964), gingival index (GI; Loe and Silness, 1963), probing depth (PD; Polson *et al.,* 1980) and clinical attachment level (CAL; Ramfjord, 1967) as the distance from the bottom of the pocket to the gingival margin and the cementoenamel junction (CEJ), respectively. The clinical measurements were obtained using a University of Michigan "O" probe with William's markings and the measurements were rounded up to the nearest 0.5 mm. The deepest point of baseline defects was included in the calculations. Routine diagnostic non-standardized periapical views using intraoral size 2 dental films were recorded by the long cone paralleling technique and holders using an x-ray unit operating at 70 kV, 10 mA, and 0.8-second exposure time. To avoid the unstable alveolar crest level, the linear distances from CEJ to the base of the bony defect, representing the rIBD component level, were measured from digital radiographs. Initial cause-related therapy and clinical measurements were performed by a single experienced calibrated examiner who was not involved in the study in any other way (SMH). 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.

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; the coin was flipped each time by the same individual (ADR) assigned immediately before surgery to either the MPM group (15 sites) or the OM group (15 sites). All surgeries were performed by the same operator (AYG). The surgical treatment phase was initiated only if the subject had a full-mouth dental plaque score of less than one. Surgical procedures were accomplished as described in detail by Gamal and Iacono (2013). A mucoperiosteal flap was elevated using intrasulcular incisions under local anesthesia. Debridement of all inflammatory granulation tissue from the intrabony defect was performed until a sound, healthy bone surface was obtained. The teeth were thoroughly root planed. For MPM samples, membrane perforations were prepared just before surgery using a custom-made 2 mm diameter pin and 2 mm perforated acrylic template with a coronal occlusive rim of about 3 mm (*Figure 1*). Inter-perforation spaces were determined to be not less than 2 mm in order to avoid loss of membrane stiffness. Collagen membranes were hydrated in sterile saline, trimmed according to the template prepared for each defect, and adapted over the defects in such a manner that the entire defect and $\geq$ 2 to 3 mm of the surrounding alveolar bone was completely covered to avoid membrane collapse within the defect. The membranes were extended supracrestally
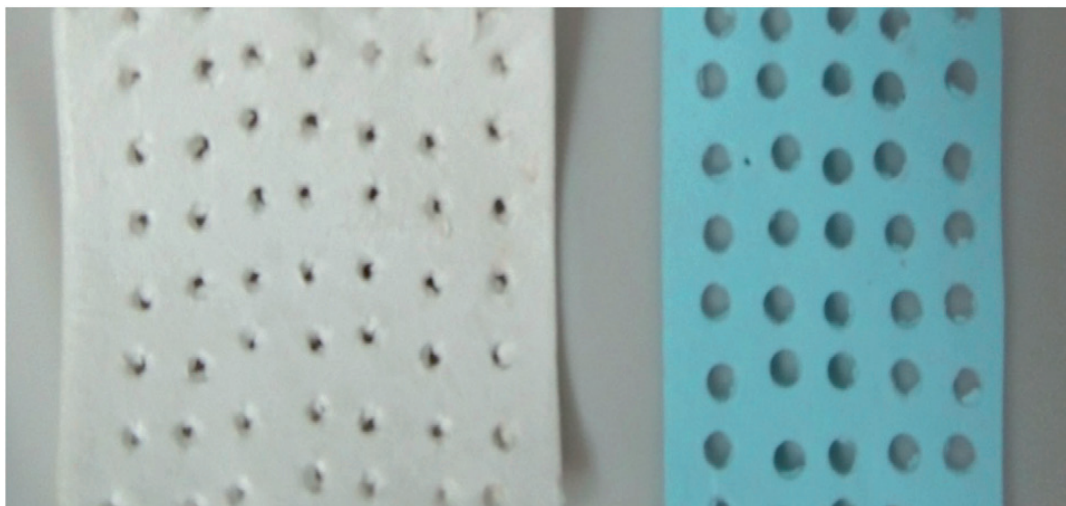
Figure 1. Template for membrane round-hole-pattern perforations (right) and perforated membrane (left).

1 mm below the CEJ to ensure optimum gingival connective tissue involvement in supracrestal wound healing. Collagen membranes were simply adapted in place according to the surgical protocol of the manufacturer without suturing. The mucoperiosteal flap was coronally positioned covering the entire membrane and sutured with a non-resorbable suture. No periodontal dressing was applied. In a separate visit, the selected OM sites underwent occlusive membrane coverage of the intrabony defects. All patients received oral and written postoperative instructions. Patients were prescribed amoxicillin (500 mg) every 8 hours for 1 week. Subjects with allergies to amoxicillin and derivatives were prescribed clindamycin (300 mg) every 8 hours.

Plaque control effort was supplemented by rinsing with chlorhexidine (0.12% chlorhexidine hydrochloride) for one minute three times daily for 2 weeks. The patients were instructed to refrain from tooth brushing and interdental cleaning was avoided at the surgical areas during this time. Sutures were removed 14 days postoperatively and recall appointments for observation of any adverse tissue reaction and oral hygiene reinforcement were scheduled every second week during the first 2 months after surgery. One month after surgery, 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. Supportive periodontal maintenance including oral hygiene reinforcement and supragingival scaling was performed during each recall appointment. Clinical and radiographic measurements were reassessed at 3, 6, and 9 months after surgery by a blinded calibrated investigator (MA).

Gingival crevicular fluid sampling and quantitative measurement of BMP-2

To avoid irritation, samples were obtained 1 day following surgery and after individuals had fasted overnight and between 8:00 AM and 10:00 AM. Using micropipettes (5 μL), GCF samples were collected (Sueda *et al.,* 1969) by a single examiner (MA) who was masked to the attribution of the sites to MPM or OM. Following the isolation and drying of the selected site with cotton rolls, a Fisher brand disposable micropipette was placed intrasulcularly at the mesio-facial line angle of the selected site to a maximum depth of 2 mm below the margin.

Micropipettes were left in place until 5 μL of fluid was collected. GCF samples were collected at day 1 and 3, 7, 14, 21 and 30 days after therapy and diluted in saline solution (50 μL) for BMP-2 level evaluation. Samples were labeled, carried in a dark container and kept at -80° C until tested. BMP-2 in the GCF samples was measured using a human BMP-2 enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. This assay uses an antibody specific for human BMP-2 coated on a 96-well plate.

Data analysis

The primary efficacy parameter for the study was gingival crevicular fluid BMP-2 level at 1, 3, 7, 14, 21 and 30 days. Secondary efficacy parameters included clinical and radiographic measurements at 3, 6 and 9 months after surgery. Data were presented as mean and standard deviation (SD) values. Data were explored for normality using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Data showed non-normal (non-parametric) distribution, so the Mann-Whitney U test was used to compare between the two groups. The Wilcoxon signed-rank test was used to study the changes

Table 1. The means ± standard deviation values of BMP-2 concentrations (pg/mL) in the groups treated with modified perforated membranes (MPM) and occlusive membranes (OM).

| Period | Group | | p - value |
	MPM	OM	
1 day	406.1 ± 122.6	43.5 ± 24.8	0.032*
3 days	223.9 ± 111.6	66.8 ± 8.7	0.038*
7 days	221.6 ± 43.4	72 ± 14.8	0.045*
14 days	143.6 ± 97.8	76.5 ± 3.1	0.197
21 days	193.9 ± 152.8	65.2 ± 3.2	0.199
30 days	92.9 ± 66.4	81 ± 22.5	0.886

*$p \leq 0.05$ (Mann-Whitney U test)

Table 2. Pre-surgery clinical measurements (mean ± standard deviation).

Characteristics	MPM (n = 13)	OM (n = 13)
PD	5.8 ± 0.3	6.1 ± 0.4
CAL	4.3 ± 0.3	4.1 ± 0.3
rIBD	6.6 ± 0.4	6.7 ± 0.3
PI	0.3 ± 0.01	0.4 ± 0.03
GI	0.2 ± 0.02	0.2 ± 0.04

MPM, modified perforated membranes; OM, occlusive membranes; PD, pocket depth (mm); CAL, clinical attachment level (mm); rIBD, relative intrabony defect depth (mm); PI, plaque index; GI, gingival index. *$p \leq 0.05$ (Mann-Whitney U test)

by time within each group. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with statistical software.

Results

During the course of the study, all patients experienced uneventful postoperative healing in all of the experimental and control defects. All patients completed the study and tolerated the surgical procedures well. No site had to be eliminated and no cases of clinically opened flap dehiscence or infection were detected. Minimal swelling of soft tissues surrounding the operated areas was observed during the early days of healing. Nevertheless, membrane exposure was a common event in both groups. It was observed at 2 to 3 weeks after surgery with minimal inflammation in five of the OM-treated sites and four of the MPM-treated sites. It was decided to include their records in the data analyses. Two patients did not continue their follow-up visits for sample collections because they relocated. As a result, 13 of 15

OM and MPM treated sites completed the study. Bony wall treated defects were distributed as follows: MPM, three predominately 2-wall and ten predominately 3-wall defects; OM, four predominately 2-wall and nine predominately 3-wall defects.

Table 1 illustrates the mean ± standard deviation (SD) values and results of Mann-Whitney U test of the BMP-2 concentrations in the GCF collected from sites treated by MPM and OM at different sampling times. BMP-2 concentrations peaked in the MPM samples obtained during the early postoperative days (days 1, 3 and 7) and were statistically significantly different than those in OM samples. BMP-2 levels decreased gradually in the samples obtained at days 14, 21 and 30 in both groups. In spite of the higher levels of BMP-2 levels in the MPM test group at 14, 21 and 30 days, there were no significant differences between the two groups.

A summary of the defect characteristics 4 weeks pre-surgically using the mean ± SD for the appropriate clinical measurements for both groups is provided in *Table 2*. No statistically significant differences were found preoperatively between MPM and OM groups with respect to soft and hard tissue measurements. All GI and PI scores were within clinically healthy parameters. The defects had deep PDs (5.8 ± 0.3 mm for the MPM group and 6.1 ± 0.4 mm for the OM group), and were associated with deep rIBD (6.6 ± 0.4 mm for MPM and 6.7 ± 0.3 mm for OM). Similarly, CAL was 4.3 ± 0.3 mm and 4.1 ± 0.3 mm for MPM and OM sites, respectively. *Table 3* shows the mean defect characteristics of both groups during different observation periods. The mean PI and GI were initially low; they remained unchanged by 3, 6 and 9 months for both groups. There were no statistically significant differences between the initial and 3-, 6- or 9-month values or between the groups (*p* > 0.05). Target teeth were free of gingival inflammation and plaque before surgery and at the end of the study. Patients were kept under a strict maintenance program,

Table 3. Chronological changes in clinical parameters.

Characteristics	PD			CAL		
	3 months	6 months	9 months	3 months	6 months	9 months
MPM	2.6 ± 0.7	2.4 ± 0.4	2.3 ± 0.3*	1.8 ± 0.4	1.6 ± 0.6*	1.4 ± 0.4*
OM	3.1 ± 0.5	2.9 ± 0.6	3.7 ± 0.2	2.1 ± 0.5	2.5 ± 0.4	2.6 ± 0.5
p value	0.094	0.078	0.024	0.093	0.035	0.024

Characteristics	rIBD			PI			GI		
	3 months	6 months	9 months	3 months	6 months	9 months	3 months	6 months	9 months
MPM	3.3 ± 03*	3.4 ± 0.5*	3.2 ± 0.6*	0.6 ± 0.4	0.4 ± 0.3	0.6 ± 0.3	0.8 ± 0.4	0.6 ± 0.4	0.7 ± 0.3
OM	4.4 ± 0.5	4.6 ± 0.7	4.2 ± 0.4	0.8 ± 0.4	0.6 ± 0.5	0.7 ± 0.4	0.6 ± 0.3	0.9 ± 0.6	0.8 ± 0.5
p value	0.033	0.037	0.041	0.67	0.45	0.36	0.65	0.56	0.36

MPM, modified perforated membranes; OM, occlusive membranes; PD, pocket depth (mm); CAL, clinical attachment level (mm); rIBD, relative intrabony defect depth (mm); PI, plaque index; GI, gingival index. *$p \leq 0.05$ (Mann-Whitney U test)
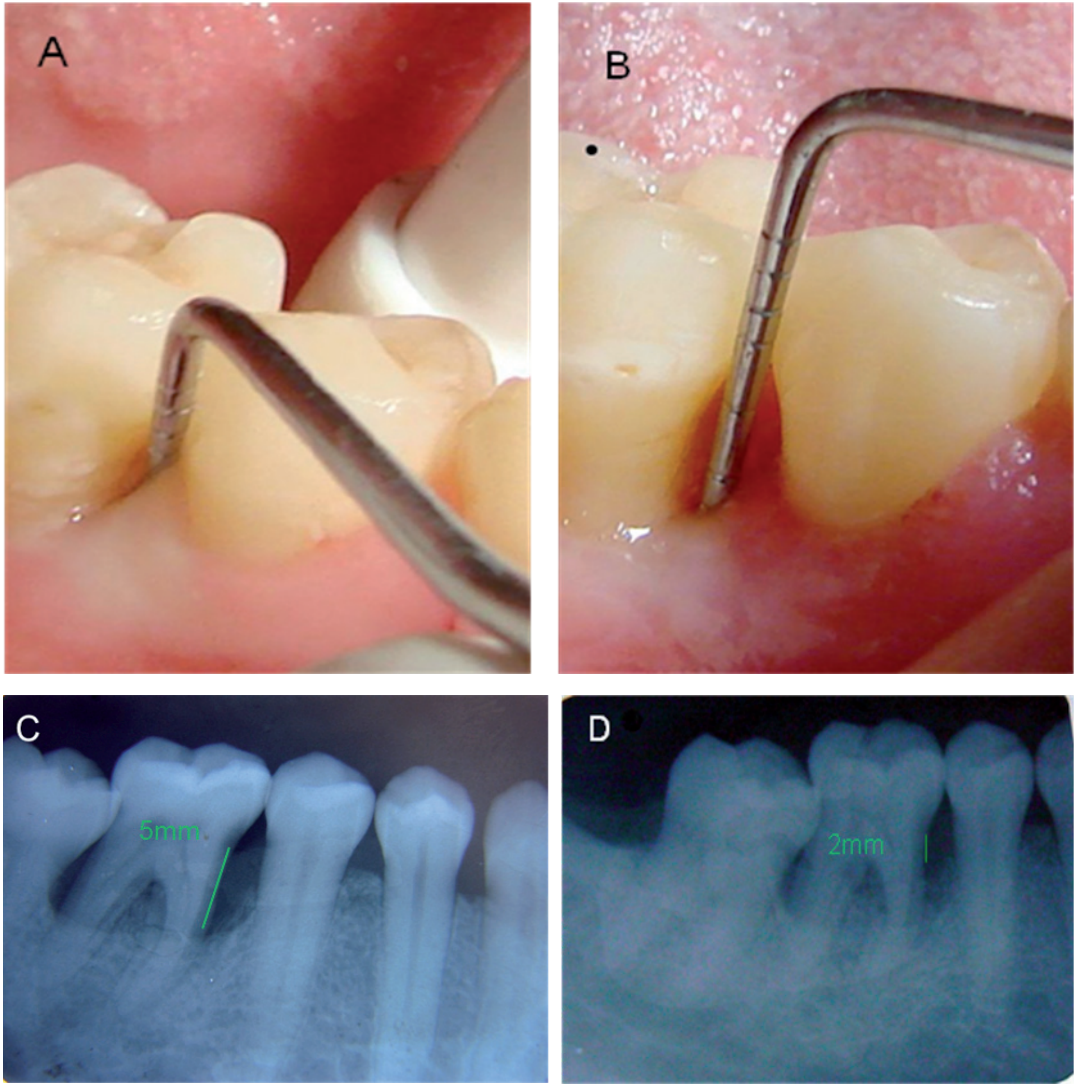

Figure 2. Clinical and radiographic views of the initial and 9-month follow up for a modified perforated membrane-treated deep intrabony defect related to the mesial root of a lower right first molar. A) 7 mm initial probing pocket depth (PPD); B) 2 mm 9-month post-operative PPD; C) initial 5 mm relative intrabony defect radiograph; D) 2 mm post-operative relative intrabony defect radiograph

and the overall plaque accumulation was minimal. By the end of the study, the MPM-treated group showed a statistically significant improvement in PD reduction and clinical attachment gain compared with the OM control group. Similarly, rIBD appeared to be significantly reduced in MPM-treated sites compared with that of the OM group (*Figure 2*).

Discussion

The main objective of using guided tissue membranes is to prevent soft tissue invasion into the periodontal defects through the use of occlusive stiff materials. Small membrane perforations and wide inter-perforation areas were suggested to keep the membrane rigid that could be easily occluded by a blood clot, providing a membrane that is mechanically obstructive for soft tissue invasion and at the same time biologically permeable for cells and mediators through fibrin clot-occluded perforations. Because the MPMs used in the present study employed bovine collagen membranes, an accepted biomaterial that does not require preclinical documentation, it was decided to initiate studies on the clinical and biochemical values of membrane perforations. It has been decided to further evaluate the positive clinical outcomes using animal models that will include immunohistochemical staining of mesenchymal stem cell markers to test our hypothesis that gingival and/or periosteal mesenchymal stem cells may selectively pass, along with gingival fibroblasts, through the perforated membrane and enhance periodontal regeneration. Our recent human trial reported improved clinical outcomes of perforated membranes as a suggested way to enhance the contribution of such cells in periodontal regeneration when compared with GTR procedures using OM (Gamal and Iacono, 2013). The hypothesis was that if the collagen is perforated, this could induce periodontal tissue regeneration in a dual direction; first in the remaining periodontal structures below the membrane, and secondly in the periosteum and gingival fibroblasts with their associated mesenchymal stem cells above the membrane. The present study is the first to evaluate the biologic effects of membrane perforations on periodontal healing. Because a direct correlation could exist between the number of cells and the available released growth and differentiation factors, the level of GCF BMP-2 following the use of perforated and occlusive membranes could reflect the number of cells releasing them.

In the present study, glass micropipettes with an internal diameter of 1.1 mm were used for the collection of GCF samples, where the fluid collection takes place through capillary action. Micropipette sample collection seems to be ideal for evaluating the released BMP-2 at different time periods because it provides an undiluted sample of "native" GCF whose volume can be accurately assessed. The use of filter paper was avoided because of the possible non-specific attachment of BMP-2 to filter paper fibers with associated false level reduction. GCF flow, with its physical protective effects of flushing the pocket, is considered an excellent undispersed medium for evaluating the released BMP-2 at different time periods. The selection of the intrabony defect type is another factor that helps in maintaining BMP-2 for accurate evaluation for its availability and release pattern. We decided to start GCF collection a day after surgery because samples collected immediately after surgeries were usually contaminated with blood. In both the MPM and OM groups, plaque control was optimal and mean gingival index scores were < 1. There were no statistically significant differences in PD, CAL, and rIBD. Therefore, the GCF flow rate was nearly consistent and BMP-2 release and subsequent containment could be under the same circumstances.

Analysis of the GCF in the present study revealed that BMP-2 levels were significantly higher at 1, 3 and 7 days after surgery in the MPM group as compared with the OM group. Levels were markedly reduced at 14, 21 and 30 days in both groups, with non-significantly higher levels for the MPM-treated group. These findings are demonstrated in previous growth factors studies in which guided tissue membranes appear to obstruct the chemotactic effect of the growth factor on periosteal pluripotential mesenchymal cells (Canalis *et al.,* 2003; Zhao *et al.,* 2003). Mechanical injury was also found to upregulate BMP-2, as well as BMP-2 signaling in human cartilage explants (Dell'Accio *et al.,* 2006). The initial low BMP-2 level that was reported in the OM-treated group suggests that occlusive membranes could act as a barrier, reducing diffusion of biologic mediators into the defect area. The higher initial BMP-2 level that was found under perforated membranes could be attributed to either direct gingival and periosteal released mediator flow through membrane perforations, or cellular migration into the defect area through membrane perforations, with subsequent enhanced mediator availability. These findings suggested that, during the early stages of healing, occlusive membranes could alter the physiologic growth and differentiation factor levels at the defect site, while membrane perforations could allow for such levels to reach periodontal defects at a physiologic level. Zhao *et al.* (2003) reported that BMP-2 decreased mRNA levels of bone sialoprotein and type I collagen dose-dependently (10-300 ng/mL). At low doses, up to 100 ng/mL, BMP-2 had no effect on transcripts for osteocalcin and osteopontin, whereas at 300 ng/mL, BMP-2 greatly increased expression of these two genes. These data reflect the diverse responses of periodontal cells to BMP-2 and highlight the necessity to consider the need to maintain physiologic mediator levels in designing predictable regenerative therapies.

The significant reduction of BMP-2 levels at 14, 21 and 30 days is supported by preclinical studies that have shown that bone formation initiated by rhBMP-2 is a self-limiting process. This self-limiting process is caused by several factors, including the presence of BMP inhibitors in the surrounding tissues and a negative feedback mechanism that functions at the molecular level (Jortikka *et al.,* 1997; Gazzerro *et al.,* 1998). The actions of BMPs are tightly regulated by natural inhibitors, such as follistatin, matrix Gla protein (MGP) and noggin. These BMP antagonists can bind to BMPs, thereby inhibit the binding of BMPs to their signaling receptors. The non-significant differences in the levels of BMP-2 between the two groups that were reported at 14, 21 and 30 days could be attributed to partial disintegration of the occlusive membranes at these time periods, which allowed for free passage of the growth factors.

Conclusions

Within the limits of the present study one can conclude that perforated collagen membrane coverage of periodontal defects is associated with a significant initial increase in GCF levels of BMP-2. This finding suggests that occlusive membranes could act as a mechanical barrier, reducing the amount of biologic mediators of the surrounding overlying tissues from reaching the defect. Further investigations are necessary with other mediators of growth and differentiation to confirm these data with larger sample sizes. Levels of bone regeneration may be evaluated by cone beam 3D dental imaging devices for further confirmation of the clinical perforation values. The suggested periosteal and gingival mesenchymal stem cellular penetration into periodontal defects through membrane perforations needs to be further investigated.

Acknowledgments

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