

Quantitative Analysis of Interradicular Bone Regeneration after Use of Modified Perforated Collagen Membrane versus Occlusive Membrane in the Management of Grade II Furcation Defects in Dogs

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Abstract: ***Aim of the work:**the aim of this study is to find out the changes in the percentage of the bone surface area in the regenerating interradicular bone which take place in association with using occlusive or modified perforated collagen membranes in management of grade II furcation defects in dogs. **Materials and Methods:** A total number of four adult clinically healthy mongrel dogs were included in the current study. Sixteen grade II critical-sized furcation defects were surgically created in lower premolars. In all four dogs, defects on the right quadrants were managed by β -Tricalcium phosphate (β -TCP) bone graft and occlusive collagen membrane and defects on the left quadrants were managed by β -TCP bone graft and modified perforated collagen membrane. **Results and conclusions:** The obtained data revealed that modified perforated membrane had a positive better role in the regenerating of interradicular bone as revealed by significantly greater value of mean percentage of bone surface area which has taken place in association with it. ($P < 0.05$)*

Keywords: Occlusive membrane, modified perforated membrane, guided tissue regeneration, grade II furcation defect, dogs

1. Introduction

Periodontitis is a disease of the periodontium characterized by irreversible loss of connective tissue attachment and supporting alveolar bone[1]. As the destruction of the periodontium progresses apically, the furcation of multirooted teeth is exposed, leading to an irreversible condition known as furcation involvement[2].

The treatment of furcation defects is a complex and difficult task. It often compromises the success of periodontal therapy[3]. In the past, several techniques have been proposed to treat periodontally involved molar furcation, and thus improve their prognosis. Various regenerative procedures have been tried with the aim to obtain furcation closure[4].

Successful regeneration of periodontal furcation defects is defined clinically as the complete elimination of horizontal and vertical defect components by bone fill. Histologically, successful furcation fill is characterized by periodontal regeneration, defined by the formation of new bone, new cementum and new periodontal ligaments over previously exposed root surfaces[4].

Attempts to treat furcation lesions have led to therapies ranging from nonsurgical periodontal therapy, such as scaling and root planning, to surgical flap debridement, root

resection, hemisection, regenerative therapy and attempts with tissue engineering[5].

New treatment modalities for osseous defects have been introduced during the last two decades, with the prime objective of enhancing the regeneration of the periodontal tissue. These include the use of bone substitutes and guided tissue regeneration (GTR) using membrane techniques[5, 6].

According to *Machtei* and *Schallhorn*, the GTR procedure is the treatment of choice for Class II furcation defects[7]. The use of augmentation materials in addition to a membrane may enhance the regenerative outcome in the treatment of furcation defects[8]. Several membranes were proposed to provide isolation of the defect against gingival soft tissue invasion[9]. There is an increase in the use of resorbable membranes for reason that a second-stage surgery is not required for membrane removal[10].

Collagen membrane is one of the most commonly used resorbable membranes, as it is the basic structural unit of connective tissue. Also it has the ability to aggregate platelets. Collagen membranes have been found to have the property of clot stabilization, wound stability, space provision [11] and epithelial cell exclusion which are important factors determining tissue regeneration when a barrier technique is used[12].

Researchers suggested that gingival connective tissue

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cells lack the potential for regeneration[13, 14]. However, later studies have reported that gingival CT cells may also contribute to the regenerative process[15].

Recently, a new population of stem cells from human gingiva, gingival mesenchymal stem cells (GMSCs) that exhibit clonogenicity, self-renewal and multipotent differentiation capacities were isolated. Therefore, isolation of the wound area from this important source of stem cells that have the capacity to differentiate into any other CT elements appears to be a loss of a great source of regeneration in periodontal defects that showed a limited regenerative power[15]. The concept of porous guided tissue membrane has been tested recently as a modality that could stimulate bone formation of critical-sized bone defects[16].

Membrane perforations could allow for gingival stem cells and periosteal cells to take part in supracrestal regeneration. In addition, Placement of a perforated membrane could allow for more flap stability through membrane pores—gingival CT integration from one side and membrane pore-clot integration from the opposing side[15].

Hence, it seemed interesting to investigate the effect of perforated membrane regarding the bone surface area in the regenerating inter-radicular septum in case of management of advanced grade II furcation defects.

2. Materials and Methods

The materials that used in this study included:

1. Collagen membrane [BioTECK, Italy]:
 - Type I equine collagen, 25x25x0.2mm made from Achilles tendon of horses.
 - Resorbable and biocompatible.
 - Supplied in a sterile vial as one membrane in double PETG blister pack.
2. β -Tricalcium phosphate (β -TC) [Bio RESORB® Classic Sybron Implant solutions, Germany]
 - It's pure β -Tricalcium phosphate (β -TCP), 500-1000 μ m in size and 1g in weight.
 - There is no addition of other mineral phase such as hydroxy apatite.
 - Materials supplied in a sterile single usage vial.

Method

A comparative study was carried out on a total of 4 male mongrel dogs (*Canis familiaris*), about 17-24 month old, weighting approximately 18-24 kg were included in the study. All dogs were clinically healthy. The mandibular third and fourth premolars (P_3 , P_4) were selected for this study.

The study was approved by the research ethics committee, Faculty of Dentistry, Alexandria University. The study included two groups

Group I: (study group) consisted of 8 surgically created critical-sized grade II furcation defects in the buccal surface of the left mandibular premolars (P_3 , P_4) that were managed by β -TCP bone graft and modified perforated collagen membrane (MPM).

Group II : (Control group) consisted of 8 surgically created critical-sized grade II furcation defects in the buccal surface

of the right mandibular premolars (P_3 , P_4) that were managed by β -TCP bone graft and occlusive collagen membrane (OM group).

Surgical procedure

The animals were anesthetized by intramuscular injection of a combination of 0.1 ml ketamine hydrochloride and 0.05 ml xylazine hydrochloride for each 100g body weight. Sulcular incisions were made and mucoperiosteal flaps were raised buccally at the mandibular P_3 and P_4 on either side of the jaw. Four grades II critical-sized furcation defects were created in each dog; the bone removal was performed using rotary burs with copious irrigation using sterile saline. The bone defects were measured using periodontal probe with Williams's markings to certify that the class II furcation defects were 5mm in vertical component and 4mm in horizontal component. The right side defects were managed by β -TCP bone graft and occlusive collagen membrane and the left side defects were managed by β -TCP bone graft and modified perforated collagen membrane. The membrane perforations were prepared just before surgery using a custom-made plastic template by rubber dam punch forceps, leaving a coronal occlusive rim about 2mm (**Figure 1**). The membranes were trimmed according to the template, hydrated in sterile saline and adapted over the defects in such a manner that the entire defect and about 2-3 mm of the surrounding alveolar bone were completely covered to avoid membrane collapse within the defect.

Finally the membranes were simply adapted in place without suturing. The adhesion of membranes to bone and root surface eliminated the need for suturing the membranes. Flaps were placed to its original position and closure of the wound area was performed with interrupted suturing, using 2-0 silk suture. The animals were sacrificed according to the following order: two animals at 4 weeks post-surgically and two animals at 8 weeks post-surgically. Scarification was done with an intravenous overdose injection of anesthesia (Concentrated sodium thiopental).



Figure 1: Modified perforated membrane

Postoperative care

All animals received intramuscular 1 gram of ampicillin in the first time just after surgery then mixed with dog's food for seven days. Non-steroidal anti-inflammatory drugs were also given intravenously just after surgery. The dogs were placed on soft diet throughout the postoperative period to reduce the possibility of local trauma to the site of operation. The animals were observed daily for the first week for presence of infection or signs of inflammation.

Euthanization of animals

Dogs were sedated by intramuscular injection of a combination of 0.1 ml ketamine hydrochloride and 0.05 ml

xylazine hydrochloride for each 100 gram of bodyweight. Dogs were euthanized with intra-cardiac injection of xylazine+ ketamine. The lower jaws were dissected out then each jaw was bisected into two halves and the specimens were preserved in 10% formalin solution and decalcified in 8% of trichloroacetic acid then processed to obtain specific serial sections to be evaluated and to be used for histomorphometric analysis using image J 1.46r software. The parameters of interest were measured for test and control groups after 4 and 8 weeks of treatment. Measurements were finally statistically analyzed.

Histomorphometric analysis of the percentage of the surface area of the formed bone in the created defects:

1. Three sections of tissue from different seven standardized depths were used to choose from for quantification from each tissue block in the different groups.
2. One photograph was taken from the best of three sections using the same magnification power for all photographs and containing the interradicular region between the two roots of the tooth, parts of PDL and parts of the adjacent two roots, so that 28 photographs were used for quantification in each group.
3. A rectangle with standardized dimensions was drawn on the photographs on the desired area to be measured using the image J program.
4. The surface area of this selected region was measured by choosing region of interest (ROI) manager, from tools from analyze and the measurement was recorded.
5. The surface area occupied by the bone marrow, parts of the PDL and adjacent two roots of the tooth were selected using the wand tracing tool and the measurement was recorded.
6. The latter measurement was subtracted from the measurement of the whole rectangle (total surface area). Its percentage to the total surface area was calculated.

Statistical Analysis

The mean value of the percentage of bone surface area was calculated in each group. Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. The used tests were:

1. **Student t-test:** for normally quantitative variables, to compare between two studied groups.
2. **Paired t-test:** for normally quantitative variables, to compare between two periods.

3. Results

Clinical Observations

All animals tolerated the surgical procedures well. Healing was uneventful. Following surgery, no adverse reactions such as postoperative infection were noted. No exposure or marginal tissue recession was observed in the teeth of both groups.

Results of histomorphometric analysis

Data obtained from the histomorphometric analysis of the mean percentage of the surface area of the formed bone in

the created defects in all groups revealed a statistically significant increase in the study groups (MPM groups) when compared with their relative control groups (OM groups) at both follow-up intervals.

After four weeks the mean percentage of the bone surface area value in the MPM groups was 50.57±7.66 which is higher than that in the OM groups which was 43.11±4.98, the data revealed a statistically significant difference between both groups (**P<0.002**). [Table 1] (Graph 1)

After eight weeks the mean percentage of bone surface area value in the MPM groups was 74.20±5.89 and it was higher than its value in their relative OM groups, it was 62.66±12.43. The data at this follow-up period revealed a statistically significant difference between both groups (**P<0.002**). [Table 2] (Graph 2)

Similarly, the obtained data revealed an increase in the mean percentage of bone surface area in all group between both follow-up intervals of the study. In the MPM groups the mean of the percentage of the bone surface area value was 50.57±47.66 at four weeks and increased up to 74.20±5.89 after eight weeks creating a statistically significant difference between the two study intervals (**P<0.001**). [Table 3] (Graphs 3).

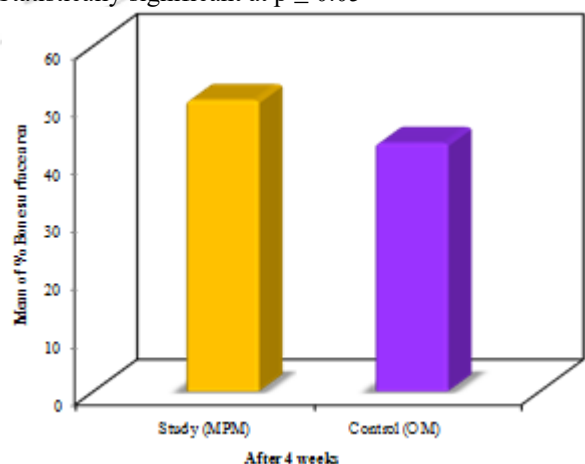
In the OM groups the mean percentage of bone surface area value was 43.11±4.98 after four weeks and increased up to 62.66±12.43 after eight weeks creating a statistically significant difference between the two study intervals (**P<0.001**). [Table 4] (Graph 4)

Table 1: Comparison between the two studied groups according to % of bone surface area after 4 weeks

	After 4 weeks		t	P
	Study (MPM) (n=18)	Control (OM) (n=18)		
% Bone surface area				
Min. – Max.	40.31 – 66.15	30.96 – 53.16		
Mean ± SD.	50.57 ± 7.66	43.11 ± 4.98	3.466*	0.002*
Median	47.47	43.14		

t: Student t-test

*: Statistically significant at p ≤ 0.05



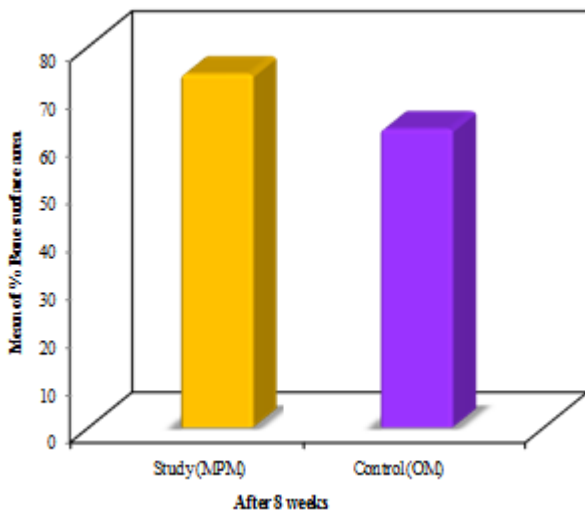
Graph 1: Bar representation of comparison between the two studied groups according to % of bone surface area after 4 weeks

Table 2: Comparison between the two studied groups according to % of bone surface area after 8 weeks

	After 8 weeks		t	P
	Study (MPM) (n=18)	Control (OM) (n=18)		
% Bone surface area				
Min. – Max.	63.85 – 85.96	40.13 – 79.76		
Mean ± SD.	74.20 ± 5.89	62.66 ± 12.43	3.560*	0.002*
Median	74.49	61.80		

t: Student t-test

*: Statistically significant at $p \leq 0.05$



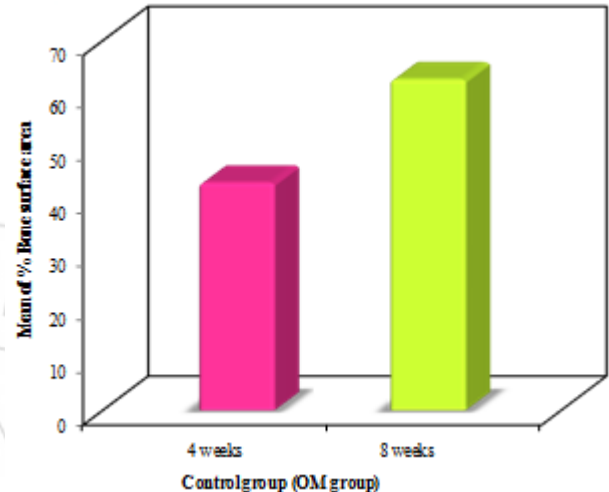
Graph 2: Bar representation of comparison between the two studied groups according to % of bone surface area after 8 weeks

Table 4: Bone surface area in control group during the two study intervals

	Control group (OM group)		t	P
	4 weeks (n=18)	8 weeks (n=18)		
% Bone surface area				
Min. – Max.	30.96 – 53.16	40.13 – 79.76		
Mean ± SD.	43.11 ± 4.98	62.66 ± 12.43	6.202*	<0.001*
Median	43.14	61.80		

t: Paired t-test

*: Statistically significant at $p \leq 0.05$



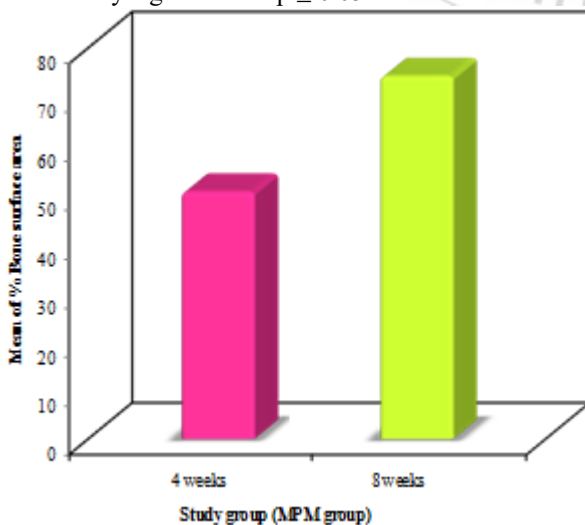
Graph 4: Bar representation of bone surface area in control group during the two study intervals

Table 3: Bone surface area in study group during the two study intervals

	Study group (MPM group)		t	P
	4 weeks (n=18)	8 weeks (n=18)		
% Bone surface area				
Min. – Max.	40.31 – 66.15	63.85 – 85.96		
Mean ± SD.	50.57 ± 7.66	74.20 ± 5.89	9.321*	<0.001*
Median	47.47	74.49		

t: Paired t-test

*: Statistically significant at $p \leq 0.05$



Graph 3: Bar representation of bone surface area in study group during the two study intervals

4. Discussion

Regenerative periodontal therapy aims to predictably restore the supporting periodontal tissues of the tooth that have been lost due to periodontal disease or dental trauma. Grade II furcation defects with their unique anatomy pose a special regenerative challenge[17]. The placement of a barrier membrane over the denuded root surfaces and the debrided periodontal defect has been shown to exclude epithelial down growth and allow periodontal ligament and alveolar bone cells to repopulate the isolated space selectively[18]. *Karring et al.*[19] found that GTR represents the well-documented regenerative procedure for obtaining periodontal regeneration in grade II furcation defects.

Both groups in the present study were managed by a collagen membrane (in the study group it was perforated) and β -TCP bone graft. Combining membrane with bone substitutes prevents the barrier from collapse and thus ensure space maintenance[20]. Besides that, graft particles aggregation serve as a barrier that inhibits soft tissue cell migration into the defect[21,22].

Collagen membrane (CM) selection was based on the fact that collagen is the principal component of connective tissue and provides structural support for tissues throughout the body. In addition, CMs exhibit chemotactic function for gingival fibroblasts and osteoblast adhesion activity[23]. It shares in common with all resorbable membranes the fact that they do not require a second surgery for retrieval, the

single-step surgical procedure will preserve bone and will be more economical and comfortable for the patient[24].

Guided tissue membrane applications protect the blood clot or the clot blended with graft material and provide the defect area with the necessary elements required for regeneration. Supracrestal periodontally affected components are usually lacking the regenerative power because of their anatomic limitations as non-contained defects bordered by epithelial-covered gingival CT from one side and a periodontally affected avascular root surface from the opposing side. Complete isolation of the supracrestal part of the defects with occlusive membrane coverage will eventually lead to root surface epithelialization[15].

The use of the perforated barrier membranes is thought to allow gingival CT cells and periosteal cells to repopulate the supracrestal part of the root surface. In the absence of epithelium via the occlusive collar, supracrestal healing will eventually occur by connective attachment to the root surface. Perforated barrier membranes were reported to open channels that allow for gingival stem cells and periosteal cells to take part in the regeneration.[15] Also they permit the growth and differentiation factors to pass from cells in the periosteum and gingival CT and augment regeneration[25].

In the current study, healing was uneventful; no adverse reactions such as postoperative infection were noted following surgery and no exposure or marginal tissue recession was observed in the treated teeth of the animals of both groups.

The results of histomorphometrical analysis revealed a substantial increase in the mean percentage of new bone surface area in MPM-treated defects compared to those in the defects treated by OM. The results of this analysis revealed a statistically significant increase in the mean percentage of new bone surface area in the study groups as compared to control groups ($p < 0.05$) at both follow-up intervals of the study.

The findings of this study correspond relatively to that obtained by *Gamal et al.*, [15] who conducted a clinical study which revealed enhanced clinical outcomes with using of modified perforated collagen membrane compared to that observed in association with use occlusive membrane in GTR procedure.

The pronounced periodontal regeneration in the MPM group could be explained on the basis that the use of perforated membrane could enable participation of periosteal cells and gingival mesenchymal stem cells (GMSCs) or their biological products of the growth differentiation factors in GTR procedure. The periosteal cells have been shown to have significant regenerative potential[26]. *Steiner et al.* [27] indicated that periosteum was able to form alveolar bone, cementum and periodontal ligament when it was transplanted into periodontal defects. Besides that, the GMSCs exhibited a multipotent differentiation capacities[28], they exhibited the potential to differentiate into osteogenic, adipogenic and chondrogenic lineages[29] and display chemotactic properties similar to immune cells

in response to tissue insult and inflammation[30]. The GMSCs may have an important role in enhancement of periodontal tissue regeneration following surgical injury.

Moreover, the growth and differentiation factors from cells in the periosteum and gingival CT could pass through the membrane perforations and augment regeneration. Such increase in the growth factors (GFs) levels could be considered an indirect factor for increased gingival fibroblast and GMSCs transmigration to the defect area through the membrane perforations which in turn enhance the periodontal tissue regeneration. This is in agreement with *Gamal et al.* [25,31] who reported that the use of MPM was associated with significantly higher gingival crevicular levels of bone morphogenic protein (BMP), platelet-derived growth factor (PDGF-BB) and vascular endothelial growth factor (VEGF).

Hence isolation of the wound area from this important sources of such cells and growth factors using occlusive membrane may limit the regenerative potential of GTR procedure.

So the histomorphometrical findings in the present study confirmed the ability of the perforated collagen membrane to successfully guide new attachment and enhance the periodontal regeneration in comparison to the occlusive one.

5. Conclusion

In conclusion the modified perforated membrane enhanced the regenerative outcomes compared with occlusive one in GTR. These results may be attributed to the passage of cells and their biological mediators from periosteum and overlying gingival connective tissue into the periodontal defects.

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