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Periodontal regeneration of dehiscence defects using a modified perforated collagen membrane. A comparative experimental study

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ABSTRACT

Placement of occlusive barrier membranes in guided tissue regeneration procedures is thought to deprive the wound area from the regenerative capacity of periosteal and gingival mesenchymal stem cells. The current study was performed to determine the effect of perforated collagen membranes in enhancing periodontal tissue regeneration when compared to conventional occlusive barriers. Sixteen critical-sized dehiscence defects (4 × 5 mm) were surgically created in the mandibular canine teeth of eight dogs, bilaterally. Eight defects were managed by β -Tricalcium phosphate (β -TCP) alloplast and modified perforated collagen membrane, the contra lateral defects were managed by β -TCP alloplast and occlusive collagen membrane. Dogs were sacrificed after one and two months. Defect sites were dissected, fixed and processed for histologic examination. Both test and control groups resulted in complete periodontal regeneration including; alveolar bone, cementum and periodontal ligament. However thicker, denser and more organized bone trabeculae with higher maturation rate and significantly higher bone surface area were noted in the perforated membrane group. Hence it was concluded that perforated membranes enhance periodontal regeneration probably by allowing periosteal and gingival mesenchymal stem cells to participate in the regenerative procedure.

1. Introduction

Periodontal disease results in irreversible loss of connective tissue attachment and supporting alveolar bone. Various therapeutic modalities have been attempted to accomplish periodontal regeneration, including guided tissue regeneration (GTR), the use of bone grafts either alone or in combination with barrier membranes, and application of various growth factors and morphogens [1–3].

Placement of a barrier membrane to cover debrided periodontal defects in GTR procedures was proved to exclude epithelial down-growth and allow selective repopulation of the isolated space with periodontal ligament and alveolar bone cells [4,5]. However, it has been debated that barrier membranes deprive the wound area from the regenerative potential of the periosteum, including progenitor cells and biologic mediators [6]. It was also reported that tissue occlusion was not a crucial requirement for GTR as periodontal regeneration including bone and cementum formation with functionally oriented periodontal ligament occurs in the presence of space provision with or without tissue occlusion [7–9].

Moreover, gingival mesenchymal stem cells (GMSCs) have been

lately isolated from gingival connective tissue. Besides their self-renewal, and multipotent differentiation capacities [10,11], they have unique immunomodulatory and anti-inflammatory properties. GMSCs contain neural-crest-derived stem cells N-GMSCs beside the mesoderm-derived stem cells M-GMSCs. N-GMSCs have an increased capacity to differentiate to neural cells and chondrocytes, and to modulate immune cells when compared to M-GMSCs [12]. These findings signify the role of GMSCs in tissue regeneration.

Aiming to enable periosteal and gingival stem cells to participate in GTR procedures, Gamal and Iacono [13] introduced a new perforated collagen membrane. They demonstrated improved clinical outcomes when these modified perforated membranes (MPM) were compared to traditional cell occlusive ones. They also suggested that growth and differentiation factors from cells in the periosteum and gingiva could traverse membrane perforations and therefore enhance regeneration [14].

The current study was performed to evaluate histologically and histomorphometrically the possible effect of (MPM) in enhancing periodontal regeneration of surgically created dehiscence defects.

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2. Material and methods

This comparative study was conducted on a total of eight adult male mongrel dogs (*Canis familiaris*), about 17–24 months old, weighing approximately 18–24 kg. The lower canine teeth, bilaterally, were selected for the study.

2.1. Materials

β -TCP (500-1000 μ m BIORESORB[®], Implant Direct Sybron International, Germany) bone alloplast. Both cell occlusive and modified perforated collagen membrane (MPM) were Type I equine collagen, 25x25x0.2 mm (BioTECK, Vicenza, Italy)

Perforations were made according to the technique described by Gamal and Iacono [13]. Prior to surgery, membrane perforations (0.5- to 1-mm diameter round holes) were prepared via a rubber dam punch forceps and by the aid of an acrylic template leaving a coronal occlusive rim of approximately 2–3 mm.

2.2. Methods

The Ethical Committee of Alexandria University, which includes the institutional experimentation committee, approved the research protocol (IRBNO:00010556-IORG0008839).

The study included two groups, each comprising eight surgically created critical-sized dehiscence defects on the buccal surfaces of mandibular canines.

Group I: (Study group) defects were managed with β -TCP alloplast and modified perforated collagen membrane.

Group II: (Control group) defects were managed with β -TCP alloplast and occlusive collagen membrane.

2.2.1. 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 followed by raising mucoperiosteal flaps buccally at the mandibular canine area on either sides of the jaw. Two critical-sized dehiscence defects (4 × 5mm) were created in each dog. Bone removal was performed using rotary burs with copious irrigation using sterile saline (Fig. 1). The right side defects were managed by β -TCP alloplast and modified perforated collagen membrane and the left side defects were managed by β -TCP alloplast and occlusive collagen membrane (Fig. 2).

Collagen membrane perforations were prepared just prior to surgery. The membranes were trimmed and adapted over the defects to cover the entire defect and about 2–3 mm of the surrounding alveolar bone to prevent collapse of membrane within defect area. Flaps were

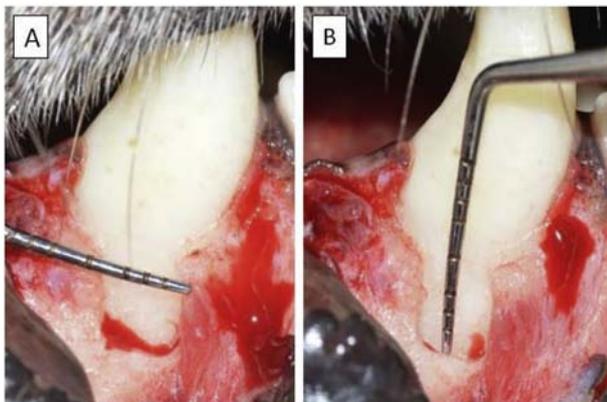


Fig. 1. (A and B) Photograph showing the creation of critical-sized dehiscence defect measuring 4 × 5mm, respectively.

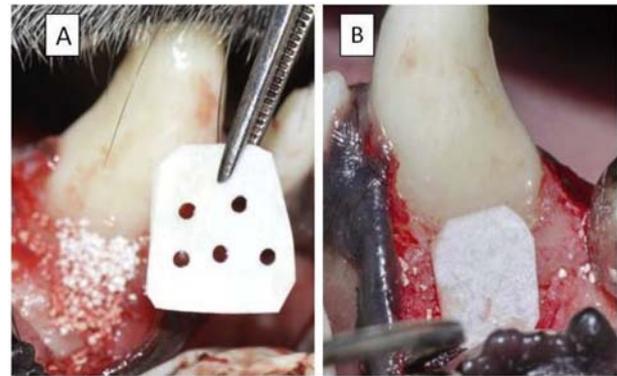


Fig. 2. Photographs showing (A)- Modified perforated membrane (B)-Occlusive collagen membrane, used to cover alloplast –filled critical –sized dehiscence defects in the right and left mandibular canines, respectively.

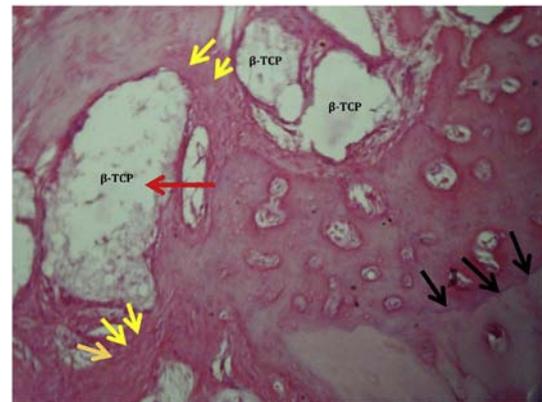


Fig. 3. Light micrograph (LM) showing one month healing defect treated with β -TCP and perforated collagen membrane with different degrees of bone maturation. Dense collagen sheets (yellow arrows) intervening more mature bone are noted. The junction between old and new bone can be detected (black arrows) and possible remnants of β -TCP particles (red arrow). H&E x100.

placed in their original position and closure of the wound area was performed with interrupted suturing, using 2-0 silk suture (Ethicon, Johnson & Johnson, Somerville, NJ).

2.2.2. Postoperative care

All animals received antibiotic Ampicillin (1gm) (Eipico Co., 10 th of Ramadan City, Egypt) intramuscular in the first day after surgery, then mixed with dogs' food for seven days. Non-steroidal anti-inflammatory (Meloxicam DELTA PHARMA Factory Industrial Zone B4,10th of Ramadan City, Egypt) was administered as a single intravenous injection of 0.4 mL/10 kg bodyweight before surgery. It was also given intravenously just after surgery. The dogs were placed on a soft diet all through the postoperative period to reduce the risk of local trauma to the operation site. The animals were observed daily for the first week for signs of infection or inflammation.

The animals were euthanized at 1 and 2 months, post-surgically with an intravenous overdose of anesthesia.

2.2.3. Specimen preparation

Jaw segments including experimental teeth, investing bone and surrounding soft tissue were then placed in a fixative of 10% buffered neutral formalin, and decalcified. Sections were cut bucco-lingually through the entire mesio-distal plane of the teeth using a rotatory microtome and stained with hematoxylin and eosin and Gomori trichrome stain.

2.2.4. Quantitative analysis of bone surface area

The bone surface area represents the density of the bone inside the defect. Three photographs were taken of each of 5 sections (total, 15 photographs) at the same magnification of tissue from different standardized depths and used for quantification. Image J program was used to measure the area occupied by bone in a standardized rectangular region of interest (ROI) after subtracting the area occupied by marrow spaces, and their mean was calculated.

2.2.5. Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution of variables, Student t-test was used to compare two groups for normally distributed quantitative variables, while Paired t-test was assessed for comparison between two periods for normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

3. Results

3.1. Clinical observations

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

3.2. Histologic results

Histologic examination of treated dehiscence defects in both the study and the control groups revealed a clear process of cell and capillary proliferation and differentiation, bone matrix formation, followed by mineralization of bone matrix to replace the β -TCP particles.

3.2.1. One month healing defects

Group-I treated with MPM showed newly formed bone trabeculae with high cellular activity and large trapped osteocytes, surrounded by dense collagen fibrous tissue aggregates (Fig. 3). Remnants of the β -TCP particles can be seen throughout the defect with bone maturation just adjacent to the particles. The junction between the newly formed bone and the native bone can be noticed merging the newly formed bone to the old bone (Fig. 3). Other samples showed mature thick bone trabeculae with less remaining bone graft particles (Fig. 4). Remnants of the collagen membrane were seen covering the newly formed well organized dense bone trabeculae, newly formed bone attained different degrees of stain with remodeling lines indicating active bone formation (Fig. 4). The PDL fibers appeared well organized with abundant blood supply and involved in establishing the tissue formation in a regenerative fashion. (Fig. 5).

3.2.2. At the two months healing period

In the MPM group bone quality was nearly all of the mature type and comprised both cancellous and compact varieties with denser and thicker bone trabeculae throughout the entire defect length. The healing defect showed less connective tissue fibers and residual bone graft particles. (Fig. 6).

3.2.3. At one-month healing period

Group II defects treated with occlusive collagen membrane showed a considerable degree of bone maturity. The newly formed bone showed similar pattern of union with the native bone. However, bone trabeculae appeared less organized and thinner than those of the MPM group with wider marrow cavities. (Fig. 7). Also more remaining alloplast particles and more intervening connective tissue was noted indicating a slower regenerative process.

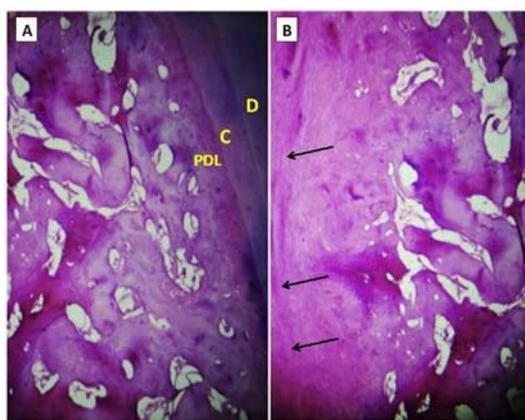


Fig. 4. (A) LM of another sample of one month healing in defect managed with alloplast and perforated collagen membrane showing; dentine (D), regenerated cementum (C) and regenerated (PDL) adjacent to newly formed irregular bone with different degrees of maturation intermingled with dense collagen fibers and viable capillaries. (B) More superficial area of the defect showing persistent remnants of the collagen membrane adjacent to the defect area separating bone from gingival tissues (arrows). H&E x100.

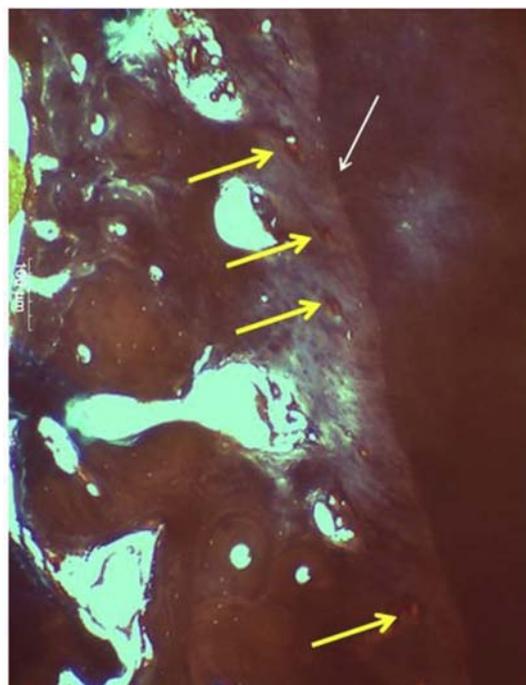


Fig. 5. LM of bone defect managed with MPM one month post-operative showing mature bone formation with new osteones adjacent to the tooth surface, periodontal ligament fibers are seen inserted into the cementum (white arrow) with high cellular activity and rich blood supply (yellow arrows). Gomori Trichromex400.

3.2.4. At two months healing period

defects treated with the occlusive membrane showed comparable bone maturity as that seen with MPM, especially at the apical part of the defect. However, more marrow cavities were noted, and graft particles being replaced by new bone formation were observed (Fig. 8).

3.3. Quantitative results of bone surface area

Histomorphometric analysis of the mean percentage of surface area of the formed bone in the created defects in both the study and the control groups showed a statistically significant increase from one to

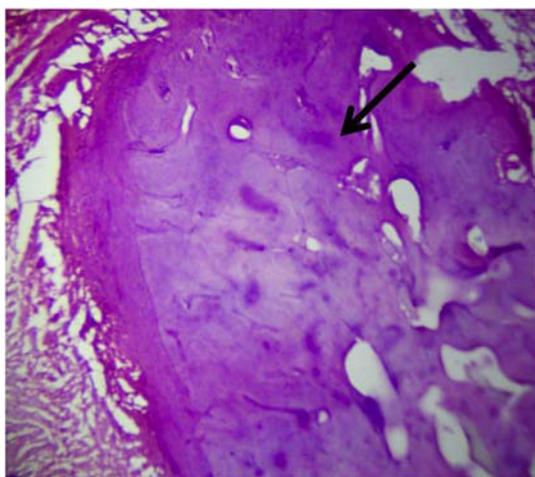


Fig. 6. LM of healing bone defect at two months post operative managed with MPM at the coronal part of the defect showing thick dense bone trabeculae with few marrow spaces. H&E x100.

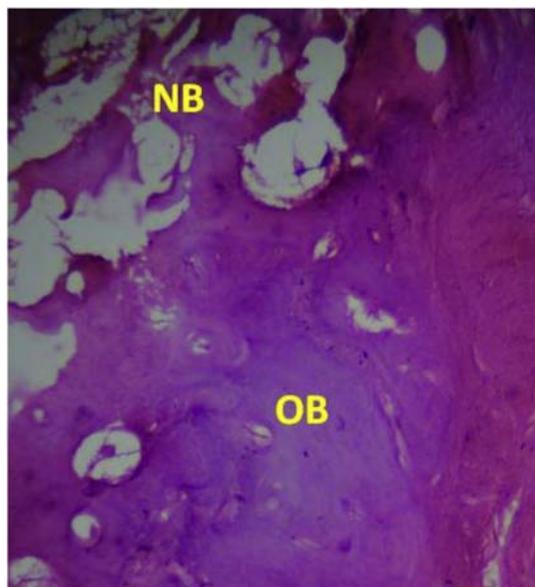


Fig. 7. LM of one month healing defect of the control group showing new bone formation (NB) with irregular bone trabeculae at the coronal part of the defect and remnants of β -TCP particles. Note the difference in maturation and quality from the old bone (OB).

two months follow up periods ($p < 0.001$). The study group treated with the MPM showed significantly higher bone surface area (70.9 ± 5.9 and 82.5 ± 6.5) when compared to the control group (44.5 ± 6.6 and 73.2 ± 6.5) at one and two months periods, respectively ($p < 0.001$). (Table 1).

4. Discussion

The gingiva and oral mucosa are usually characterized by reduced inflammation, rapid re-epithelialization, and fetal-like scarless wound healing. This is unlike healing of skin wounds in which scar formation is quite common [15,16]. This observation was explained by the isolation of (GMSCs) from gingival connective tissue [10,11]. Apart from the well-established self-renewal and multipotent differentiation properties, mesenchymal stem cells exhibit both immunomodulatory and anti-inflammatory roles. GMSCs are capable of suppressing peripheral blood lymphocyte proliferation and induce the expression of several

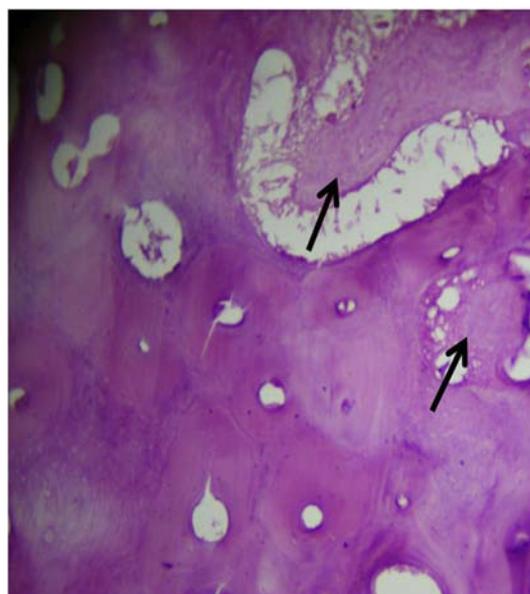


Fig. 8. LM of two month healing defect of the control group showing mature bone with new osteons formation and new bone forming in place of the resorbing β -TCP particles (black arrows) H&E. x200.

Table 1

Comparison between the two studied groups according to percent of bone surface area.

Percent of bone surface area (%)	Test	Control	p_1
1 month	70.9 ± 5.9	44.5 ± 6.6	$< 0.001^a$
2 month	82.5 ± 6.5	73.2 ± 6.5	$< 0.001^a$
p_2	0.001^a	$< 0.001^a$	

p_1 : p value for Student t-test for comparing between two studied groups.

p_2 : p value for Paired t-test for comparing between 1 month and 2 month in each group.

^a Statistically significant at $p \leq 0.05$.

immunosuppressive factors including IL-10, inducible nitrous oxide synthase (iNOS), and cyclooxygenase 2 (COX-2) in response to the inflammatory cytokine interferon -gamma IFN- γ [17].

In an attempt to allow GMSCs to participate in regeneration of periodontal defects, this study was performed using MPM in critical-sized dehiscence defects in a canine model. The perforated barrier membranes are thought to allow GMSCs and periosteal cells to repopulate the isolated defect area. Leaving about 2 mm of the coronal part of the membrane non-perforated (occlusive collar) helped exclude the gingival epithelium. In the current study, there was complete periodontal regeneration including; PDL, cementum and bone in both studied groups. Considerable defect fill with new bone formation with varying degrees of maturity was achieved. Periodontal ligament fibers were observed inserted in the regenerated cementum and bone as Sharpey's fibers revealing restoration of the attachment apparatus. The effect of GTR on periodontal regeneration has been well-documented. Placement of a barrier membrane over denuded root surfaces has been shown to exclude epithelial down growth and allow periodontal ligament and alveolar bone cells to repopulate the isolated defect space [18–20].

Noteworthy was the rapid bone maturation in the MPM group revealed by denser, thicker and more organized bone trabeculations at one month healing period. As healing proceeded, the MPM group showed almost complete substitution of the graft particles with dense mature bone. PDL in the MPM group showed more cellular activity and rich blood supply adjacent to the regenerating cementum. It has been documented that cementum contains molecules that promote

chemotactic migration, adhesion, proliferation, and differentiation of progenitor cells from the PDL. Growth factors and adhesion molecules present in cementum also activate cells of the gingiva, and alveolar bone [21–23]. Membrane perforations may have facilitated these procedures; allowing the cementum to activate gingival and alveolar bone cells, and from the other side allowing the recruitment of GMSCs and periosteal cells to participate in the regenerative procedure. Periosteal cells have also been reported to form alveolar bone, cementum and periodontal ligament when transplanted into periodontal defects [23]. On the other hand, considerable regeneration was noted in the control group, however, it lagged behind the MPM one. More immature woven bone and collagen fiber aggregates were noted in the control group at four weeks, approaching a more mature pattern of regeneration at two months.

Similar findings were reported in a study by Gamal et al. [13] who obtained enhanced clinical outcomes when using MPM for treating intrabony defects by GTR. In addition to the suggested effect of gingival and periosteal cells, the authors reported that growth and differentiation factors from cells in the periosteum and gingiva could go through the membrane perforations and enhance regeneration. Analysis of the GCF in treated sites showed that BMP-2 levels were significantly elevated at 1, 3 and 7 days after surgery in the MPM group as compared with those in an occlusive membrane group [14]. Membrane perforations enable BMP-2 to reach superior local concentrations in the defect site required to induce bone formation. BMPs have strong osteoinductive activity, induce differentiation of mesenchymal cells into chondrogenic and osteogenic cells and promote osteoblast proliferation [24]. Other growth factors have also been studied in MPM treated osseous defects; where MPM coverage of periodontal defects was related to initial gingival crevicular fluid vascular endothelial growth factor (VEGF) and platelet derived growth factor-BB (PDGF-BB) up-regulation that improved the clinical outcomes of periodontal regeneration [25]. Recently, a perforated barrier membrane was also used to enhance lateral bone augmentation for implant site development, and resulted in 5.0 mm lateral bone gain [26]. When used in aggressive periodontitis patients, modified perforated collagen membrane resulted in improved defect fill when compared to standard collagen membrane [27].

Moreover, histologic evaluation of MPM was performed in experimentally created furcation defects. Similar to the results of the current study, denser and thicker bone trabeculations in the MPM treated furcation defects were noted together with a significantly higher bone surface area in the study group at four and eight weeks intervals. The authors suggested that membrane perforations aided in stabilizing the formed fibrin clot within periodontal defects through mechanical interlocking of fibrin strands with the pores, therefore allowing more membrane and clot stability [28].

Membrane perforations also serve to stabilize the flap through integration of the gingival CT from one side with the formed blood clot on the opposing side [13]. These properties are extremely critical for wound stabilization, which is an essential factor in promoting periodontal regeneration, as exceeding the tensile strength of a fibrin clot leads to tearing of the formed clot and healing with long junctional epithelium attachment [29].

Enhanced periodontal regeneration obtained in the current study was reflected by the significantly denser bone, and by the more organized bone trabeculae and faster bone maturation in defects treated with the MPM. This could be explained by GMSCs and periosteal cells passing through membrane perforations into the defect site, allowing a higher level of growth factors to pass from gingival and periosteal cells and stabilizing the healing wound.

5. Conclusion

MPM resulted in enhanced periodontal regeneration in surgically created dehiscence defects in dogs with the formation of significantly

denser bone trabeculations, more rapid bone maturation together with the formation of cementum and periodontal ligament, more than the traditional OM. Further studies with larger sample size are required to accurately analyze MPM enhanced bone regeneration. Also research to identify cells implicated in periodontal regeneration is needed to enrich our understanding of the different wound healing and tissue regeneration mechanisms.

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