

EVALUATION OF BONE AUGMENTATION UNDER PERFORATED AND NON PERFORATED CERAMIC SPACE MAINTAINING DEVICE (EXPERIMENTAL STUDY)

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ABSTRACT

INTRODUCTION: Alveolar bone deficiency is one of most common problems encountered with oral rehabilitation. Many bone augmentation techniques have been documented, one of them is augmentation by bone grafting and barrier membranes, ceramic membrane proved efficiency in guided bone regeneration due to enhanced properties.

AIM OF THIS STUDY: Evaluation of bone surface area under perforated ceramic membrane in relation to non perforated ceramic membrane.

MATERIALS AND METHODS: 14 mongrel dogs divided into two equal groups, study group used bone graft, cortical perforation and perforated ceramic membrane, control group used bone graft, cortical perforation and non perforated ceramic membrane, follow up for 12 weeks and sacrifice was done retrieving ceramic membrane with samples of new bone which histologically prepared for histomorphometric analysis.

Results: By histological evaluation more cellularity and vascularity, better trabecular arrangement and lesser granulation tissues were noted under perforated ceramic group. By histomorphometric analysis statistically significant at $p \leq 0.05$ and higher bone surface area with mean value of 40.09 ± 3.50 associated with perforated group in comparison with mean value of 19.92 ± 3.63 associated with non perforated ceramic membrane group.

CONCLUSION: Perforated ceramic membrane is comparable to occlusive one in terms of higher bone surface area, reduced granulation tissues and favorable blood supply.

KEY WORDS: Guided bone augmentation, Ceramic membrane, Cortical perforation.

RUNNING TITLE: Effect of membrane porosity on guided bone augmentation.

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INTRODUCTION

Different amounts of bone loss may be present in orofacial region as a sequel of teeth loss, trauma, infection, periodontium affection, congenital deformities and surgical procedure (1-3).

In oral rehabilitation rebuilding is a prerequisite. So various grafting materials and bone regeneration techniques have been used (4).

One of these techniques is guided bone regeneration which is when adding grafting material termed guided bone augmentation (5).

Guided bone regeneration is based on creation of a framed cavity permitting formation of new bone and preventing other tissue involvement (6-9).

Variant materials have been used as barriers for isolating and recently framing these cavities. Biocompatibility, fitting and good isolation are the requirements needed for these barriers (10).

The coupling of bone substitute and barrier is commonly used to reconstruct the bone defect in which the bone substitute maintains three dimensional scaffold to support the osteogenic cells (11).

Ceramics are non-metallic, inorganic materials that have high hardness, brittleness, poor conductivity, high melting temperature, excellent biocompatibility (11), and recently ability to be fabricated into any desired design which is space making ability (12).

Like any other body tissue a vascular blood supply is essential for new bone formation. Cytokines, growth factors, hematopoietic cells and osteoprogenitor cells will mediate new bone formation and reach the target area through the blood. The osteogenesis is the first stage of ossification (1-6).

Perforation of the cortical bone enhance blood oozing and clot formation. Endosteum which lines medullary spaces is a provider for osteogenic cells (1-6).

Periosteum is an impressive source of blood and bone forming cells, but also its outer layer is a dense fibrous layer rich in precursor cells for fibrous tissue (13).

Cortical bone perforation before bone graft commonly used as a part of guided bone augmentation, however the impact of perforation on new bone formation still in debate (1, 14).

On the other hand the competitive role in bone formation between endosteum and periosteum is also in debate (15).

The cells collected from the periosteum and bone marrow can terminate into osteoblasts in vitro, in vivo studies on the osteogenic potentials of periosteum and bone marrow are limited, the difficulty in understanding the role of different origins of cells during skeletal regeneration emerges in part from the compound structure of bone and the multiple tissues associated (16).

Some researchers suggested that more porosity of ceramic membrane is negative beyond resulting bone volume (12).

The study has been exposed to occlusive ceramic membrane and perforated ceramic one which explained the contribution of bone marrow and periosteum on bone formation.

The null hypothesis was no significant statistical difference between perforated and non perforated ceramic membrane on bone formation.

MATERIALS AND METHODS

Materials

Animals

This study was performed on 14 mongrel adult healthy male dogs 1.5-2 years age with average weight of 10-12 kg. They were housed and received their care in the faculty of agriculture, animal laboratory, Alexandria University. They have been housed 1 week before surgery under standard suitable diet, water and climate condition of well ventilated rooms. They were divided in two equal groups of 7 dogs.

- **Study group** received the perforated membrane.
- **Control group** received non perforated ceramic membrane.

Sacrification has been done 12 weeks after the operative procedure.

Ceramic membranes

Fourteen hollow dome shaped ceramic predesigned and fabricated membranes of zirconia (17) **designed and fabricated with CAD/CAM Dental Lab Alexandria** have been used.

They have inner diameter of 6 mm with height of 4mm. The thickness is 1.5 mm (17), 7 of them were perforated from the surface opposing periosteum with

standard 9 pores with diameter of 1.2mm each. (**Fig. 1A**) The other group used as a solid completely occlusive barriers. (**Fig. 1B**) The shape of the dome designed on auto CAD program and fabricated by Roland DWX52D milling machine, sum 3D program.

Tricalcium phosphate bone graft

The synthetic bioinert material act as scaffold for bone formation (Straumann group- Alexandria).

Fixation screw

Biocompatible titanium fixation screws (**Fig. 1C**) with length of 3 mm for fixation of ceramic membrane, and fixation kit (**Fig. 1D**) obtained from Straumann group- Alexandria.

Methods

Preoperative Care

- Housing of the dogs was done one week before surgery under the same diet of milk, broth and meat throughout the whole period of the study.
- Examination was done by veterinarian to exclude diseased or non suitable animals.
- The dogs fasted the night before surgery to eliminate vomiting after anesthesia.
- Ampicillin 25mg/kg (Misr company for pharma industrial) was given intramuscularly one hour before the operation.

Surgical Procedure

- All surgical procedures were conducted under sterile conditions in the animal theatre.
- Each dog was anesthetized through intramuscular injection of ketamine hydrochloride 35 mg/kg (Rotexmedica, Trit- tau, Germany), plus Debocaine 5 mg/kg (DBK pharmaceutical S.A.E ADWIC/ Eldebiky cairo, Egypt).
- The dogs operated in supine position and the surgical area washed with 2% povidone iodine. (Betadine- El-Nasr pharmaceutical chemical company, Alexandria, Egypt).
- Gingival incision by Bard Parker scalpel blade no. 15 was done from the last molar connecting with vertical incision released mesial to the last premolar in the mandible of the dog, the mucoperiosteal flap was reflected exposing the buccal surface of the body of the mandible. (**Fig. 2A**)

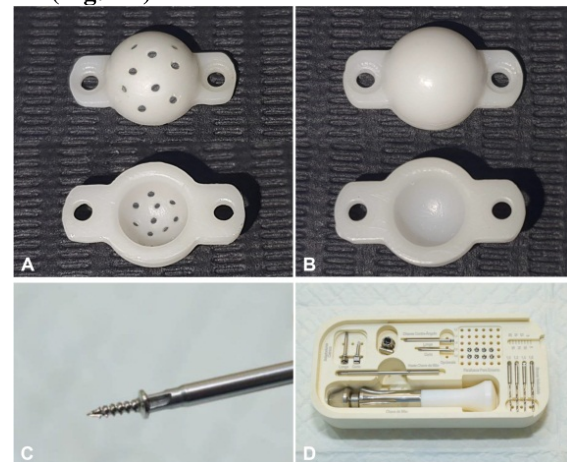


Figure (1): (A) Perforated ceramic membrane. (B) Non perforated ceramic membrane. (C) Fixation screw. (D) Fixation screw kit.

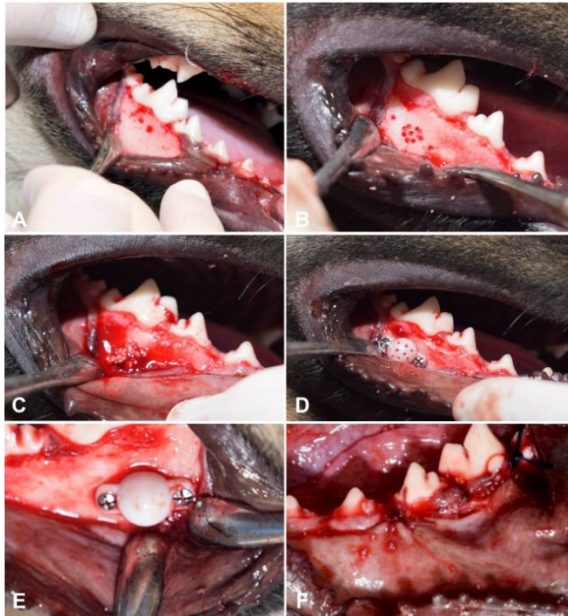


Figure (2): (A) Flap reflection. (B) Decorticated area. (C) Application of bone graft. (D) Perforated ceramic membrane. (E) Non perforated ceramic membrane. (F) Suturing.

- Rose head bur size 4 mounted on rotary hand piece was used to perforate the cortical bone equal 9 pores which accounts for 50% of the target area of the cortex. (**Fig. 2B**)
- Tricalcium phosphate was mixed with blood and adapted into the decorticated area. (**Fig. 2C**)
- Study group covered with screw retained perforated membrane. (**Fig. 2D**)
- Control group covered with screw retained non perforated one. (**Fig. 2E**)
- The surgical site was sutured using 3-0 vicryl. (**Fig. 2F**)

Post-operative phase (18)

Each dog received intramuscular ampicillin 25 mg/kg body weight every eight hours for five days, Ketorolac tromethamine 1 mg/kg (ketolac by El-Amryia Company- Alexandria, Egypt) subcutaneously every 24 hours was given as pain killer and anti-inflammatory drug to the animals for 3 days after surgery.

Glucose water was given to the animals on the first post-operative day.

The dogs kept under observation for any postoperative complication as infection, wound dehiscence or any allergic reaction. They were maintained on soft diet consisting of bread, milk and broth post-operatively.

Follow up phase

- The experimental dogs were transferred to a clean cage to be observed for the signs of infection, wound dehiscence or any complication. Any alter in coat display, dietary

habits, or actions was observed very closely (18).

- Sacrification of the dogs was done 12 weeks after surgery via euthanasia using overdose of intravenous anesthetic sodium pentobarbital which induce profound depression of the central nervous system followed by death (17).

Histological preparation (19, 20)

The specimens from each group were collected at 12 weeks from the initial surgery.

The area of bone augmentation was crossed out en bloc to retrieve membranes with newly formed bone and prepared for histological examination. (**Fig. 3**)

The preparation of the decalcified histological sections was operated according to the following steps.

Fixation by 10% neutral formalin, **Decalcification, Dehydration** of the specimens was done by immersion in a gradual increasing concentration of alcohol 50%, 70%, 90% and the absolute concentration (El Nasr Pharmaceutical Chemicals Co, Egypt). Then the samples were placed on filter papers to allow their complete dehydration.

Clearance of alcohol by xylene (Xylene, Technical grade, El Nasr Pharmaceutical Chemicals Co, Egypt).

- **Infiltration** of the samples was done with paraffin, then **Sectioning** of the samples was done by rotary microtome.
- The sections were mounted on clean slides, and then placed on a constant-temperature drying table, at about 56°C.
- The sections were deparaffinized in absolute alcohol to ensure complete removal of xylene from sections. Then they were hydrated by passing the slides through gradual decreasing concentration of alcohol 90%, 70% and 50% (two minutes each) and finally through distilled water.
- The serial sections of each specimen were then stained with Hematoxylin - Eosin stain and Trichrome stain.

Histomorphometric analysis

Computer-assisted histomorphometry was performed in order to measure the percentage of bone surface area formed in the two different groups. (**Table 1**)

Statistical analysis of the data

Data were delivered to the computer and explained using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to establish the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

The used test was: student t-test

For normally distributed quantitative variables, to contrast among two studied groups.

RESULTS

Clinical results

The entire dogs exhibited normal activity during the follow-up phase, except three dogs with moderate signs of infection which treated by previously mentioned intramuscular antibiotics and disinfecting wound by povidine solution. The dogs also showed uneven healing without abnormal reaction or wound dehiscence. About two weeks after surgical operation there was no complication.

Histological results

Histological examination of the **study group** (perforated group) revealed the following:

- The cavity was filled by mature compact bone that has higher density than non-perforated group. **(Fig. 4A)**
- The higher magnification of the compact bone exhibited osteons with high cellularity and rich blood supply existed within Haversian and Volkmann canals. **(Fig. 4B)**
- Numerous reversal lines were also seen within the formed compact bone. **(Fig. 4C)**

Histological examination of the (H & E) stained sections after 12 weeks in the **control group** (non perforated group) revealed the following:

- Filling of the entire cavity with granulation tissue, bone trabeculae and dense compact bone. **(Fig. 5A)**
- The bone trabeculae were sparse, and irregular in arrangement with poor cellularity and limited blood supply. **(Fig. 5B)**
- The formed compact bone was dense with limited blood supply.

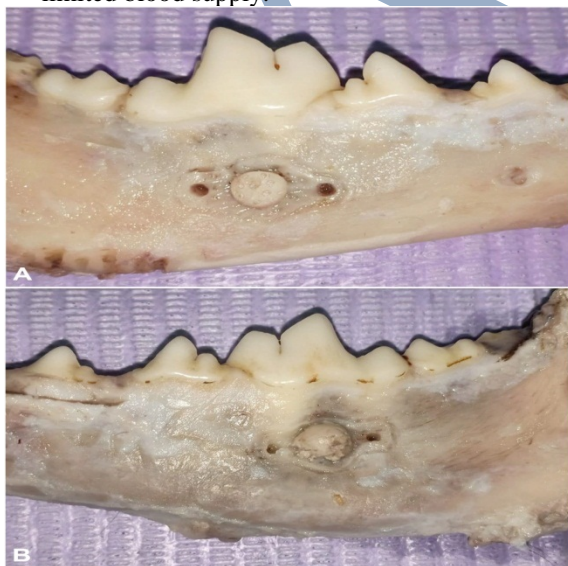


Figure (3): (A) Resected augmented perforated specimen (B) Resected augmented non perforated specimen.

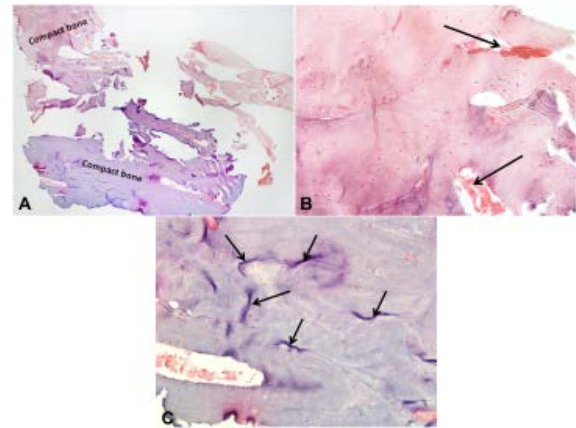


Figure (4): (A) Light micrograph shows filling of the entire cavity with mature dense compact bone. H and E X40 (perforated). (B) Light micrograph shows higher magnification of the formed compact bone with high vascularity. Note the presence of blood vessels with red blood cells within Haversian and Volkmann canals. H and EX200 (perforated). (C) Light micrograph shows numerous dark stained reversal lines existed within the formed compact bone. H and E X 200 (perforated).

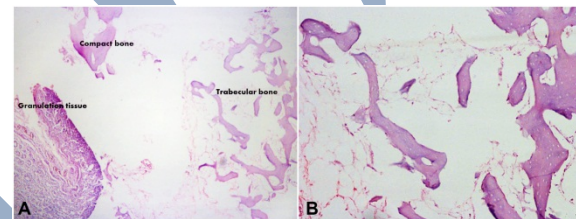


Figure (5): (A) Light micrograph shows filling of the cavity with trabecular bone, compact bone and granulation tissue H and E stain X40 (non perforated). (B) Light micrograph shows bone trabeculae with poor vascularity and cellularity (non perforated).

Statistical results

The histomorphometrical results of our study were significantly different with higher mean value of newly formed bone surface area associated with perforated membranes, it was 40.09 ± 3.50 compared with smaller value 19.92 ± 3.63 associated with occlusive membrane. (Table 2 & Fig. 6)

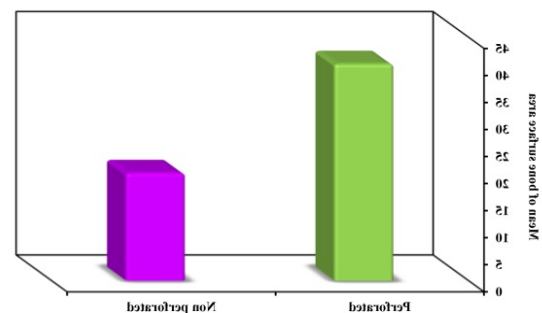


Figure (6): Contrast among the two studied groups in relation to bone surface area.

Table (1): Values of bone surface area.

Bone surface area	Bone surface area (non perforated)
39.8496	12.5812
37.0024	19.156
46.5884	20.434
39.5288	23.624
43.0188	17.5004
41.2068	23.022
35.8508	20.2552
37.6904	22.7732

Table (2): Contrast among the two studied groups in relation to bone surface area.

Bone surface area	Perforated (n = 8)	Non perforated (n = 8)	t	p
Min. – Max.	35.85 – 46.59	12.58 ± 23.62		
Mean ± SD.	40.09 ± 3.50	19.92 ± 3.63	11.320*	<0.001*
Median (IQR)	39.69(37.4– 42.1)	20.34(18.3– 22.9)		

IQR: Inter quartile range SD: Standard deviation
 t: Student t-test
 p: p value for comparing between the studied groups
 *: Statistically significant at $p \leq 0.05$

DISCUSSION

The present study was trying to conduct an ideal method of horizontal and vertical alveolar bone augmentation with illustration of the roles of different suppliers of bone regenerative elements which are bone marrow and periosteum, it is conducted to investigate the influence of perforation of ceramic membrane on bone augmentation through histomorphometric analysis for bone surface area, it is operated on 14 mongrel dogs divided equally into a study group with perforated ceramic membranes and control group with completely occluded membranes.

Dogs were elected for our study as being cost effective, mandible size closely resemble that of the human that can accommodate membrane and graft (21).

The study is consistent with many other previous studies (14, 22-24), support the need for porosity of GBR membranes to enable beneficial regenerative capacity of periosteum.

The main difference noted with histological evaluation was angiogenesis, rich blood supply and higher cellularity were noted with perforated group, mature compact bone of higher density with numerous reversal lines were also seen within the formed compact bone indicating faster healing and maturity of bone inside permeable ceramic membrane.

Limited blood supply, granulation tissue and sparse irregularly arranged bone trabeculae were the main features associated with occlusive membrane bone specimen, these features of bone limit its suitability for implant placement.

Gutta et al., (2009) (22) has been harnessed the periosteal tissues by using barrier with macroscopic holes to allow communication with periosteum, the results revealed more bone formation. In contrast to Schmid et al., (1994) (25) who claimed that the periosteum isn't necessary for bone formation consequently there is no need for permeable membrane, they contrast completely occlusive titanium membrane with opened one, better findings encountered with occlusive membranes, but the period of healing of study was 8 months which claimed to be long period for evaluation.

Lundgren et al., (1998) conducted an experimental study on rats, the membranes were group of different pores size and occlusive group, the results exhibited swifter bone formation in permeable membranes than in occlusive one at a period of 4, 8 weeks. After 12 months there was no significant differences in the results (23). In general faster healing and bone formation was detected with increased permeability in short periods evaluation (22, 23).

Colnot (2009) reported that bone marrow and endosteum cells doesn't participate in callus formation, so the periosteum is crucial for callus formation (16).

Yamada et al., (2003) conducted a study on rabbit calvaria utilizing totally occlusive and macroscopically perforated titanium domes, the obtained results after 4 and 12 weeks showed less bone formation of permeable membrane in relation to occlusive membrane which oppose results of our study (26).

Anderud et al., (2016) supported the later findings of Yamada et al., (2003) in which a conducted experimental study using ceramic domes of hydroxyapatite on a rabbit model exhibited better quality of bone accompanied with complete occlusive membrane in comparison with perforated one (17, 26).

Employment of totally occlusive membrane has raised the utilization of bone marrow and endostum and then the need for decortication and gaining access of bone marrow.

Majzoub et al., (1999) reported significant enhancement in bone formation with cortical perforation during guided bone regeneration (27).

Lee et al., (2014) notified more angiogenesis and osteogenesis with cortical perforation at early stages of healing in rabbit model but no notable difference was reported at late stages (28).

However our study was based on the previous study of Anderud et al., (2014) (19) and (2016) (17), it showed opposite results, they concluded that periosteum has no effect on bone formation under ceramic dome and then there is no need for permeability of ceramic membrane, Schmid et al., (1994) (25) and Yamada et al., 2003 (26) supported the findings of Anderud et al., (2014) and (2016).

The selected barrier in the study was ceramic membrane of zirconia due to higher

biocompatibility, enhanced soft tissue reaction (17, 19), and ability to be fabricated to any shape in horizontal and vertical direction using modern digital dentistry (29).

Development of delicate fibrous tissue layer was reported to be formed between zirconia and newly formed bone (30), which grant easier removal of membrane, it was also reported that a ceramic zirconia membranes have osteoconductive property (12). The used thickness of zirconia membrane in this study was 1.5 mm which can be decreased in future studies providing more volume for bone formation and easier soft tissue repositioning, also in future studies zirconia membrane could be replaced by ceramic hydroxyapatite membrane that can be left in bone due to closer structure of inorganic component of bone, so a second surgery for barrier removal can be prevented (17).

The field of surgery was determined opposite molar teeth to avoid mental nerve and it was midway between crestal margin of bone and inferior border of mandible to avoid injury to inferior alveolar canal. The length of fixation screw was 3 mm with 1.5 mm exhausted in the membrane and 1.5 inside cortical bone permitting safety to the roots.

The newly formed bone in the present study evaluated within 12 weeks and showed significant difference supporting perforated membranes, this difference seems to be decreased with longer periods of evaluation. So future studies can be planned with more than 12 weeks post-operative follow up.

Other variables seem to have an impact on different results of the previously discussed studies as periosteal quality which depend on properties of overall mucoperiosteal complex as thick and thin gingival biotype (31). Also degree of permeability still in debate, despite the barrier pores promote the diffusion of nutrients, oxygen and bioactive elements for bone and soft tissue regeneration (32) it has been reported that large pore size induce soft tissue cells to overpopulate regenerative area affecting quality of bone (25).

CONCLUSION

In conclusion the present study explained that the permeability of ceramic membrane was favorable in terms of better bone quality and higher bone surface area.

Conflict of interest

No conflict of interest similar to this article was announced.

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