

## **BONE MORPHOGENIC PROTEIN EXPRESSION OF INDUCED MEMBRANE USING TWO TYPES OF POLYMETHYL METHACRYLATE SPACERS IN MANDIBULAR SEGMENTAL DEFECTS: AN EXPERIMENTAL STUDY**

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### **ABSTRACT**

**Aim:** The aim of this study was to evaluate bone morphogenic protein-2 (BMP-2) expression in the induced membrane formed around mandibular segmental defect filled with 2 types of polymethyl methacrylate (PMMA) spacer over a period of 2, 4, 6 and 8 weeks, and to question whether the use of dental self cure acrylic resin spacer instead of PMMA bone cement spacer affect histological and osteopromotive characters of the induced membrane

**Materials and methods:** This study was conducted on 24 healthy male mongrel dogs. For each animal segmental mandibular resection was performed to create a critical size defect. The defect is filled with PMMA bone cement spacer in 12 animals, while for the other 12 animals the spacers were fabricated from self cure acrylic resin. 6 dogs (3 dogs from test group and 3 from control group) were scarified at 2, 4, 6 and 8 weeks postoperatively for histological and immunohistochemical analysis.

**Results:** Histological result showed similar reaction in both groups. Immunohistological results showed that 4<sup>th</sup> week represents the peak of BMP-2 expression in both groups.

**Conclusion:** This study indicated that the optimal time for reconstruction is 4 weeks after spacer insertion. It also showed that dental self cure acrylic resin can be used instead of PMMA bone cement for spacer fabrication.

**KEYWORDS:** Masquelet technique, Mandibular reconstruction, Bone morphogenic proteins, Polymethyl methacrylate.

### **INTRODUCTION**

Mandible represents a unique structure in reconstructive surgery. It acts as a framework for muscles attachment playing an important role in phonation,

deglutition and mastication<sup>(1-3)</sup>. Mandibular defects disturb mastication, swallowing, speech and impair patient's quality of life. Consequently, reconstruction surgery aims to restore continuity, contour,

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support, function and occlusion<sup>(4,5)</sup>. Segmental mandibular defects represent a challenging situation as different techniques have been introduced throughout the years. These techniques defer mainly in graft source and type, time of reconstruction, and fixation method<sup>(6-10)</sup>. The selection of the optimal technique is controversial and individually depends on different local, systemic and socioeconomic factors. These factors include defect size and site, soft tissue availability and quality, histopathology of the lesion, adjunct therapy, patient's age, medical status, desire, expectations and lifestyle<sup>(11-13)</sup>.

Autogenous bone is considered the gold standard in bone grafting procedures. It has been harvested from different donor sites either as vascularized or non vascularized graft<sup>(14,15)</sup>. Vascularized free bone grafts provide large amount of available composite tissue and superior blood supply allowing immediate reconstruction of complex and radiated defects. On the other hand, microvascular surgery is expensive, time consuming, needs experienced surgeon, longer hospital stay, and can't be tolerated in some cases<sup>(16-19)</sup>.

Non vascularized autogenous grafts are considered as a reliable method for reconstruction of lateral segmental mandibular defects less than 5-6cm with adequate soft tissue bed and cases where microvascular surgery is contraindicated or failed<sup>(20-25)</sup>. Unlike vascularized graft, non vascularized autogenous graft showed controversial results in immediate reconstruction. Traditionally, this technique is accomplished in 2 stages. In the first stage resection and fixation is performed. After 6-8 weeks, the grafting procedure is performed. This lag period aimed to allow soft tissue healing, maturation, self-decontaminate and avoiding early contamination of the graft. However, during this period the soft tissue undergo fibrosis and shrinkage leading to collapse of the defect site<sup>(26-28)</sup>.

Alain C. Masquelet introduced induced membrane technique to overcome these drawbacks. In the first stage, the defect is filled with polymethyl

methacrylate (PMMA) cement spacer and soft tissue reconstruction is performed if needed. After 8 weeks, the spacer is removed and the grafting procedure is performed<sup>(29,30)</sup>. Although, the function of this spacer initially was to create a chamber for the prospective grafting site and prevent soft tissue collapse and fibrous tissue invasion, the newly formed membrane showed high biological activity<sup>(31)</sup>.

The induced membrane was proven to be highly vascular preventing the graft resorption and improving revascularization. It also secretes growth factors as transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), vascular endothelial growth factor (VEGF), and bone morphogenic protein-2 (BMP-2)<sup>(32-34)</sup>. This biological activity of the membrane opened the field for further investigations concerning the optimal time for second stage surgery, nature of the spacer, and type of graft used<sup>(31,35)</sup>. Moreover, studies conducted to evaluate Masquelet technique are concentrated on long bones and limited studies are available to assess this technique in mandibular reconstruction<sup>(36-38)</sup>.

The aim of this study was to evaluate BMP-2 expression in the induced membrane formed around mandibular segmental defect filled with 2 types of PMMA spacer over a period of 2, 4, 6 and 8 weeks, and to question whether the use of self cure acrylic resin spacer instead of PMMA bone cement spacer affect histological and osteopromotive characters of the induced membrane.

## MATERIALS AND METHODS

This study was conducted on 24 healthy male mongrel dogs (*Canis Familiaris*) with an average weight and age of 20 kg and 18 months. All dogs were examined thoroughly and quarantined in separated cages under observation for 2 weeks preoperatively. The kennels were sprayed with 6/1000 ml Neocidal diazinone and the dogs were bathed in 1/1000 Neocidal diazinone.

Animals included in this study were injected by Ivermectin (0.1 mg/kg body weight) subcutaneously and repeated doses were injected monthly. For each animal odontectomy and alveoplasty was performed for the right side of mandible. After 4 weeks, a segmental mandibular resection was performed in the prepared area to create a critical size defect. The defect is filled with PMMA bone cement spacer (Cemex isoplastic, Tecres, Verona, Italy) in 12 animals, while for the other 12 animals the spacers were fabricated from dental self cure acrylic resin (Acrostone, Acrostone Co., Cairo, Egypt). 3 dogs from each group were scarified at 2, 4, 6 and 8 weeks postoperatively and tissues surrounding the bone defect were collected for histological and immunohistochemical analysis.

## **I. Stage I: Odontectomy and alveoplasty**

### ***a. Pre-surgical preparation and anesthesia***

Animals were starved for 12 hours and thirsted for 6 hour before surgery. Atropine sulphate (0.05 mg/kg body weight) was administrated subcutaneously 10-30 mins before surgery. A mixture of Xylazine HCL (1 mg/kg body weight) and ketamine HCL (5mg/kg body weight) was administrated IV to induction anesthesia. Maintenance of anesthesia was performed by continuous infusion of thiopentone sodium (25 mg/kg body weight in 2.5%.dilution). A combination of Penicillin (2 gm) and Gentamycin Sulphate 10% (0.5 mg/kg) were injected.

### ***b. Surgical procedure***

The surgical field was scrubbed with betadine and the animal draped in regular surgical manner. Buccal and lingual gingival incisions were performed from the first right premolar to the last right molar. Full-thickness mucoperiosteal flaps were reflected to expose buccal and lingual plates of bone. The bicrooted teeth were sectioned and extracted using lower premolar forceps in rotational movement. The alveolar ridge was trimmed using

bone rongeur and large round bur under copious amount of irrigation with normal saline. The flap was trimmed, returned into position and sutured using 2/0 vicryl suture (International Suture Manufacture company, Egypt). The opposing upper molar teeth were extracted by the same surgical procedure to avoid traumatic injury of the wound. (Figure 1)

### ***c. Postoperative care and follow up***

After recovery the dogs were returned back to their cages. A combination of Penicillin (2 gm) and Gentamycin Sulphate 10% (0.5 mg/kg) were injected every 12 hour for 7 days. All animals were kept on soft diet for 4 weeks and watched for any signs of wound infection or dehiscence. They were kept in high caloric soft diet for one week then return back to normal diet and activity. Postoperative lateral oblique radiograph (40 KV, 100 MA, 0.5 sec) was taken one month postoperatively to assess healing of the bone at the prepared side using 10×12 inch film (Eastman Kodak Company, Rochester, New York, USA).

## **II. Stage II: Segmental resection of the mandibular and insertion of the spacer**

This stage is performed 4 weeks after stage I.

### ***a. Pre-surgical preparation and anesthesia***

Animals were prepared by the same manner as stage 1 surgery. Furthermore, the submandibular region was prepared by shaving then washing with water and soap. Local anaesthesia articane hydrochlorid 4% with 1:100.000 epinephrine (Septanest SP, Septodont) was administrated. Finally, the area was disinfected with betadine.

### ***b. Surgical procedure***

Skin incision was performed 1 cm below the inferior border of the mandible. Careful dissection was carried out through platysma and subplatysmal plane till the periosteum of the inferior border of the mandible. The periosteum was then incised and

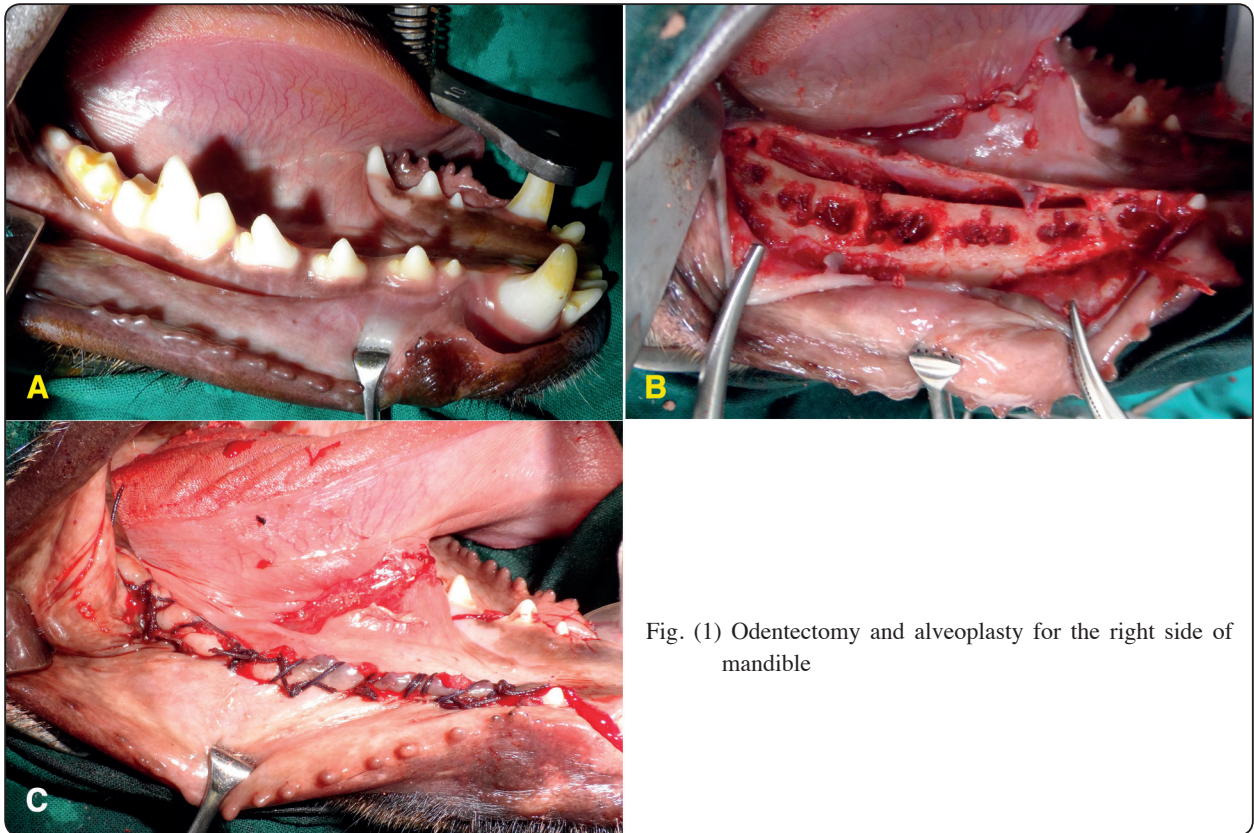


Fig. (1) Odontectomy and alveoplasty for the right side of mandible

reflected carefully (avoiding perforation of the oral mucosa) to exposure of the mandibular body, crest of the ridge and inferior border of the mandible. Reconstruction plate (6 holes 2.7 DCP plate, depuy syntheses vet, West Chester, PA, USA) was adapted and screwed to the buccal surface of the mandible. 2 cm segmental resection was performed after plate removal using sharp diamond surgical disc under copious amount of irrigation. The reconstruction plate was then fixed in proper alignment using the existent holes to keep the relation between bone segments. The bone cement spacer was fixed to the plate to fill the bony defect. Finally, the field was irrigated with normal saline and sutured in layers with vicyle 2/0. (Figures 2)

#### **c. Fabrication of the spacer**

The spacer was fabricated by mixing the PMMA bone cement (control group) or self cure acrylic resin (test) as advised by the manufacturers. The mix

was packed during dough stage in a prefabricated rubber bases mould to maintain the same shape and contour of the mandible after segmental resection. The spacer was removed from the mould after complete setting and any irregularity or sharp edges were removed. Finally, the spacer is sterilized using autoclave. The mould was fabricated by taking impression to the resected bone segment of a pilot case using rubber base impression material.

#### **d. Postoperative care and follow up**

Postoperative care and follow up were performed by the same manner as stage I surgery. The head was protected for the first two weeks postoperatively using head collar. Immediate postoperative lateral oblique radiograph was taken (40 KV, 100 MA, 0.5 sec) to check up the plate and implanted segment using 10×12 inch film (Eastman Kodak Company, Rochester, New York, USA).

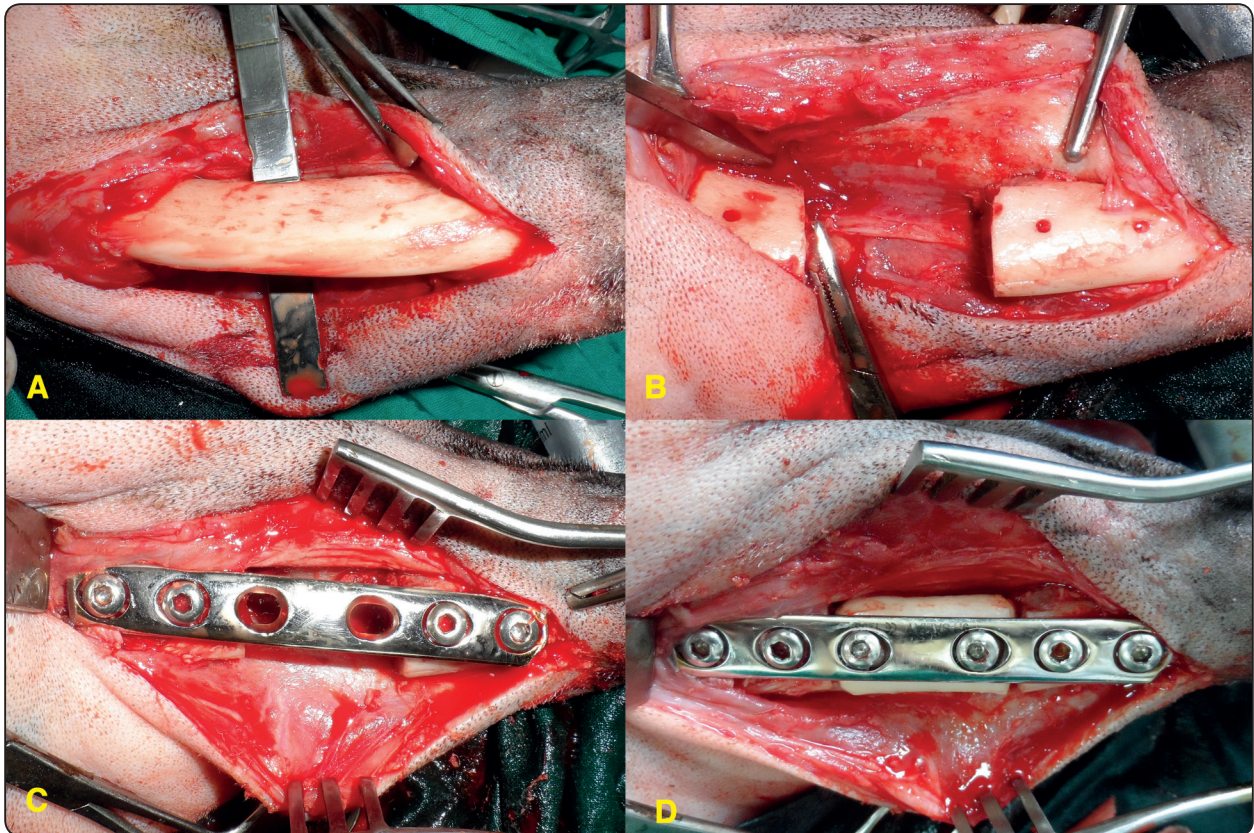


Fig. (2) Stage II (a) Mandibular body, crest of the ridge and inferior border of the mandible exposure after submandibular incision before plate adaptation. (b) Segmental resection performed after plate removal (note screw holes). (c) Reconstruction plate fixed in proper alignment using the existent holes. (d) Spacer fixed to the plate.

### III. Stage III: Animal euthanasia and specimen processing

6 dogs (3 dogs from test group and 3 from control group) were scarified using an overdose of thiopental sodium at 2, 4, 6 and 8 weeks postoperatively. The head was decapitated. Tissues surround the bone defect were collected, and specimens were fixed in formaldehyde 10% for 2 days for histological and immunohistochemical analysis.

The specimens were dehydrated using ascending alcohol, followed by clearing in xylol. Then it was embedded in paraffin wax to form blocks. The paraffin blocks were sectioned longitudinally using a microtome into thin paraffin sections of approximately 3 microns. Finally, Paraffin section slides were then processed routinely and stained with hematoxylin and eosin for histological examination. While for immunohistochemical analysis slides

were overlaid with antibody specific for BMP-2.

## RESULTS

### Clinical and radiographic results

Animals showed slight discomfort after surgical procedures (stage I and II) for 1 week postoperatively. Clinical observation showed normal mucosal healing without any signs of infection, dehiscence or bony exposure after odontectomy and alveoplasty. Correspondingly, normal healing of the extraoral wound was achieved in all animals and no intraoral dehiscence was observed after mandibular resection and insertion of the spacer. Lateral oblique radiograph showed adequate bone healing for the prepared side after stage I surgery. It also showed proper relationship between the proximal and distal stumps of the resected side, and spacer position. (Figure 3)

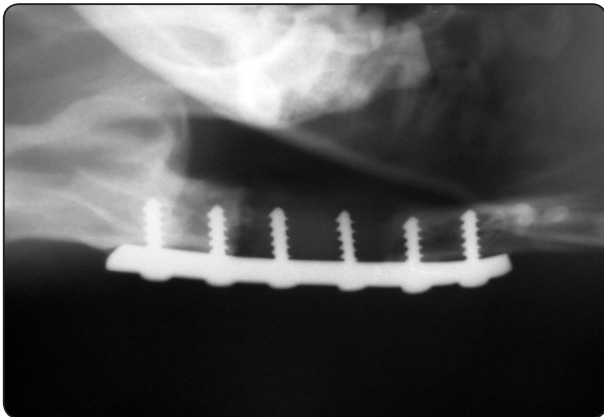


Fig. (3) Lateral oblique radiograph showing proper relationship between the proximal and distal stumps of the resected side and spacer position.

### Histological results

Microscopic examination was carried out at 2, 4, 6 and 8 weeks following spacer placement. For both groups, 2 weeks specimens showed multiple

areas of degeneration and hyalinization associated with numerous inflammatory cells infiltration and congested blood vessels representing tissue reaction toward the implanted material. After 4 weeks, specimens revealed marked decrease in area of degeneration and appearance of more dense collagen fibers accompanied with newly formed blood vessels. It also showed new lymph vessels lined by thin layer of endothelial cells, with inflammatory cells still infiltrating the tissues. While 6 weeks specimens showed marked increase in vascularity, decrease in inflammatory cells infiltration and formation of more dense properly arranged collagen fibers. Large lymphatic spaces appeared in these specimens representing cessation of the inflammatory process toward the spacer. Finally, after 8 weeks inflammatory cells disappeared, and collagen fibers became arranged and denser. (Figure 4)

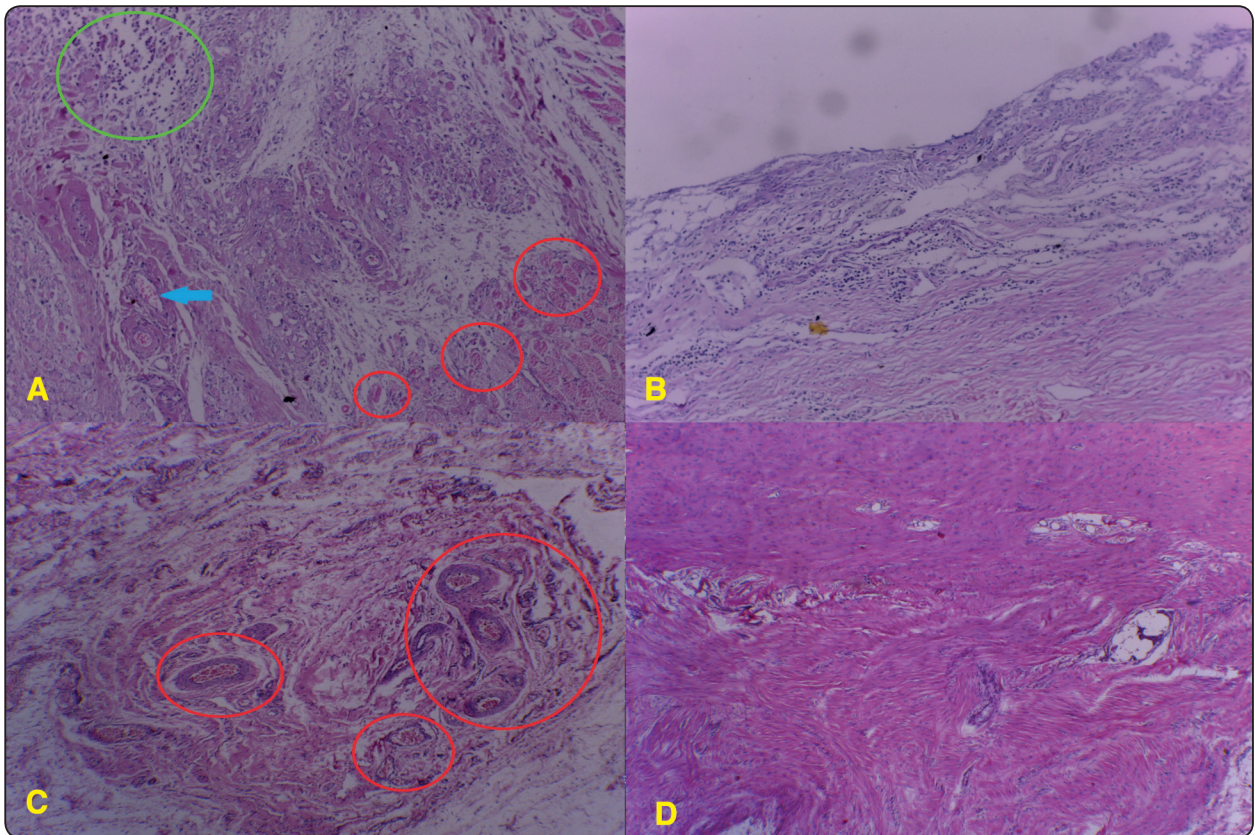


Figure 4 Histological results (a) 2 weeks: specimen showing areas of degeneration and hyalinization (red), inflammatory cells infiltration (green) and congested blood vessels (blue) (b) 4 weeks: specimens showing dense collagen fibers with inflammatory cells infiltrating the tissues. (c) 6 weeks: specimen showing marked increase in vascularity (red), decrease in inflammatory cells infiltration and formation of more dense and properly arranged collagen fibers (d) 8 weeks: specimen showing dense and properly arranged collagen fibers, and disappearance of inflammatory cells.

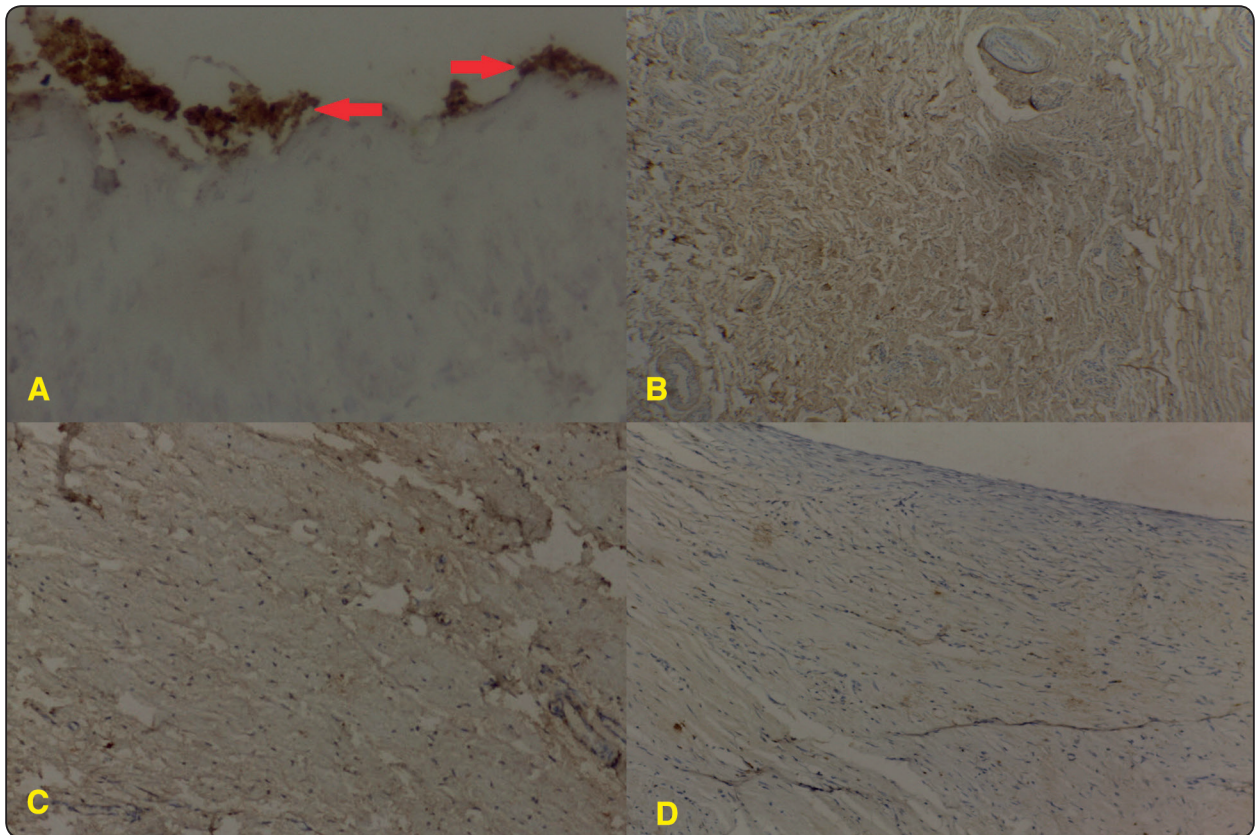


Fig. (5) Immunohistochemical results (a) 2 weeks: BMP-2 reaction limited to the surface cell layer. (b) 4 weeks: Diffused BMP-2 reaction, not limited to the surface layer. (c) 6 weeks: Diffused BMP-2 reaction but less than 4 weeks. (d) 8 weeks: diffuse faint reaction indicating the decrease in BMP-2.

### Immunohistochemical results

At 2 weeks, analysis of tissue around the spacer showed localized immunohistochemical reaction to BMP-2 limited to the surface cell layer just adjacent to the spacer. This reaction was diffused and not limited to the surface layer in 4 weeks specimens. After 6 weeks, the tissues were still sensitive to the reaction indicating the presence of BMP-2 in large amount but less than 4 weeks. While 8 weeks specimens showed diffuse faint reaction indicating the decrease in BMP-2. (Figure 5)

### DISCUSSION

Induced membrane technique has been introduced by Masquelet and over the years it gained popularity especially on long bones. However,

several questions are still unsolved and need further investigations. These queries are concentrated on the optimal time for reconstruction, optimal grafting material and finally the type and nature of the spacer used<sup>(31, 35, 39)</sup>.

In this study, odontectomy and alveoplasty were performed 4 weeks before resection to allow for proper soft tissue healing. This stage also included extraction of opposing teeth to prevent traumatic exposure of the plate and spacer. During the second stage, segmental resection was performed to create critical bone defect as indicated by Huh et al study<sup>(40)</sup> and the defect was filled by PMMA spacer.

Commercial compound of acrylic acid have been produced in 1901 by Otto Rohm. Thereafter, PMMA have been used in a variety of healthcare

applications<sup>(41,42)</sup>. Dental fixtures and denture base materials was the first medical use of this technology. In 1950s, Charnley used dental self cure acrylic resin as bone cement for total hip arthroplasty. However, clinical results were poor for mechanical and biological reasons. This urges Charnley to develop a new PMMA product which had more adaptable characteristics (Plexiglas). Over the following decades, bone cement has become widely used for orthopedic prostheses. Furthermore, various additives and modifications have been introduced to improve its mechanical and biological characteristics<sup>(43-45)</sup>.

In the current study, spacers were fabricated from bone cement in control group and dental self cure acrylic resin for test group to assess whether the use of dental self cure acrylic resin spacer instead of PMMA bone cement spacer affects histological and osteopromotive characters of the induced membrane. The spacers were fabricated outside the surgical field using a mould to omit the toxic effect of polymerization heat (exothermic reaction) and residual monomer<sup>(46,47)</sup>.

Clinical results showed normal soft tissue healing without any signs of infection or dehiscence. This result is in accordance with Zwetyenga et al and Christou et al studies performed on rabbit mandibles and sheep humeri<sup>(36,39)</sup>. Histologic evaluation of tissues around spacers showed increased inflammatory reaction in the first 4 weeks, this reaction subsided at 6 weeks and completely disappeared at 8 weeks. On the contrary, dense collagen fibers associated with newly formed blood vessels started to appear at 4<sup>th</sup> week and became arranged and denser at 8<sup>th</sup> week. This result revealed the formation of foreign body reaction fibrotic capsule around the spacer. This reaction resembles various studies performed on induced membrane technique<sup>(33,34,48)</sup>.

To date, more than 20 BMPs have been identified, and they have different functions. Regarding bone

formation, BMP-2 plays a critical role through osteoinduction and osteoblast differentiation<sup>(49-51)</sup>. Immunohistochemical analysis was performed to assess the expression of BMP-2 in the induced membrane. It showed higher BMP-2 level at 4, 6 weeks compared to 2, 8 weeks with peak level at 4<sup>th</sup> week. This result is comparable to Pelissier and Henrich studies<sup>(33,34)</sup>.

This study showed that the use of PMMA spacer after mandibular resection is useful to maintain the space for the prospective grafting material and to produce highly active biological membrane. It also showed that dental self cure acrylic resin can be used instead of bone cement for spacer fabrication. Finally, this study pointed out that the optimal time for reconstruction is 4 weeks after spacer insertion. However, this conclusion was based only on BMP-2 expression indicating the need for further studies to evaluate the presence of other growth factors in induced membrane and their role in selection of the optimal time for reconstruction.

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