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## RESEARCH ARTICLE

# THE ROLE OF INTERNAL LAYER OF PERIOSTEUM IN A NEW BONE FORMATION IN THE DYNAMIC PERIOSTEAL DISTRACTION: AN EXPERIMENTAL STUDY IN THE RABBITS

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#### **ABSTRACT**

**Purpose:** The purpose of this study is to evaluate the role of internal layer of periosteum in a new bone formation in the dynamic periosteal distraction.

**Materials and methods:** A custom-made dynamic periosteal distraction device consisted of titanium plate fixed to the lateral surface of the mandible in 10 adult rabbits. The plate was perforated in 5 rabbits (group1) and non-perforated in the others (group 2). Periosteal distraction was started 7days after placement of the periosteal distraction device. Then the device was activated at a rate of 0.25 mm every12 hours for 5 days. The animals were sacrificed after of 8 weeks of consolidation period. The specimens were then fixed, decalcified, and stained with hematoxylin and eosin.

**Results:** In group 1, Histological evaluation of control specimens showed a various amount of new bone formation with large spaces between bone trabeculae. On the other hand, a thin layer of a newly osteoid bone under the titanium mesh was detected in group 2 after 8 weeks.

**Conclusion:** The results of the present study indicated that the internal layer of periosteum has important role in bone formation in the dynamic periosteal distraction.

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## **INTRODUCTION**

Dynamic periosteal distraction osteogenesis (PDO) is a relatively new technique, and its potential for producing new bone was first reported by Schmidt et al. (2002). This method is based on the concept that tensile strain on the periosteum, which causes tenting of the subperiosteal capsule, is sufficient to produce bone formation, without corticotomy or local harvesting of the bone (Oda et al., 2009). The most recent concept of PDO based on the potential of the Periosteal Mesenchymal Stem Cells to differentiate into osteoblasts to fill the space over the underlying bone (Schmidt et al., 2002). However, other studies showed that the newly formed bone was mainly produced from the basal bone, not from the periosteum. (Zakaria et al., 2012) Sencimen et al. (2007) and Altug et al. (2011) reported that the newly formed bone by periosteal distraction is rich in interstitial fatty tissue. The role of the mesh-perforations is still a matter of debate (Al Nashar et al., 2016). In the dynamic PDO, it seems to be important to have sufficient communication between the periosteum and the underlying bone with appropriate mechanical strength against the overlying soft tissue to encourage new bone formation (Yamauchi et al., 2013). On the other hand, it has been

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reported that elevation of periosteum with collagen membrane covering the perforated titanium plate produces more new bone compared to the elevation with the perforated titanium plate alone (Saulacic *et al.*, 2012). The purpose of this study is to evaluate the role of internal layer of periosteum in a new bone formation in the dynamic periosteal distraction.

## **MATERIALS AND METHODS**

# **Experimental animals**

Ten adult male rabbits with a mean weight of  $2.4 \pm 0.35$  kg were used as the animal model. Experimental protocols were approved by University of Al Andalus University Committee of Animal Research. The animals were equally divided into two groups. In group 1 the plate of distraction device was perforated and non-perforated in group 2.

#### **Device description**

The device is consisted of a titanium plate either perforated or non-perforated. The lower part of the plate has two holes for fixation screws (3mm length and 1mm diameter mini-screws) and the upper part has one serrated hole for distraction screw (titanium elevation screw with 10 mm length and 2 mm in diameter). Fig(1)

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## Surgical procedure

All surgical procedures were performed under general anesthesia with a combination of 35 mg/kg intramuscular ketamine and 5 mg/kg subcutaneous xylazine. Local anesthesia, consisting of 2% lidocaine with 1:100,000 epinephrine was infiltrated into the lateral surface of the mandibular body. The surgical site was shaved, prepared with 10% povidone-iodine solution, and draped to maintain aseptic conditions. A 1.5-cm-long incision in the skin was made along the inferior border of the mandible, and dissection was performed through the subcutaneous and muscle layers. The periosteum was carefully elevated to expose the lateral aspect of the mandibular body and the buccal cortex was porously perforated by drilling with fissure bur. The device was placed over the perforated area and then fixed to the bone surface from one end by 2 mini screws then the periosteum was sutured covering the whole plate finally the skin flaps were sutured with 4-0 silk. Soft tissue incision of 2mm in length was made over the activation screw and then the screw was rotated 360° to elevate the plate 1mm. Postoperative analgesics included ketorolac (0.5 mg/kg by mouth) and buprenorphine (0.3 mg intramuscular). After latency period of 7 days the screw was activated to elevate 0.25 mm twice a day for 6 days. Fig (2) After healing periods of 8 weeks, animals were sacrificed by an intravenous overdose of pentobarbital sodium. The mandibular distraction areas, including peripheral soft tissues, and distraction devices were carefully removed. All resection materials were kept in a 10% neutral buffered formalin solution for at least 3 days.

Next, each distraction device was removed. The specimens were then decalcified in the formic acid solution. When sufficiently soft, tissue samples were processed and embedded in paraffin for histological examination. Standard 4–5-mm sections were prepared and transferred onto slides for each block of tissue. All slides were stained with haematoxylin and eosin and evaluated using a light microscope.

## **RESULTS**

All animals resumed normal dietary habits during the first 24 hours after the operation, and none of the animals had a weight loss during the experimental study.

**Group 1:** The histological evaluation showed a various amount of new bone formation with large spaces between bone trabeculae. The bonewas covered by a lining of osteoblasts and characterized by an increase in the number of osteocytes per unit area. The new bone is separated from the original bone by a high vascularity thin layer of connective tissue and a layer of periosteal proliferation. Fig (3,4)

**Group 2:** The histological evaluation showed a thin layer of a newly osteoid boneunder the titanium mesh, the bone was covered by a lining of osteoblasts. The new bone is separated from the original bone by a thick connective tissue which is rich in collagen fibers, cells, new blood vessels and a layer of periosteal proliferation. Fig(5,6)



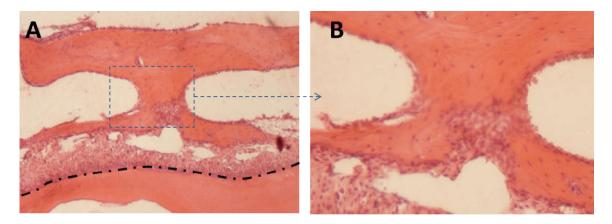


Fig.(1). The periosteal distraction device, A: before activation 1, B: at the end of activation

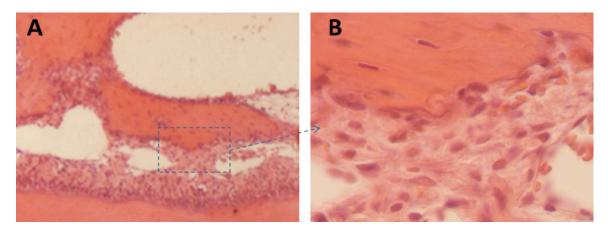




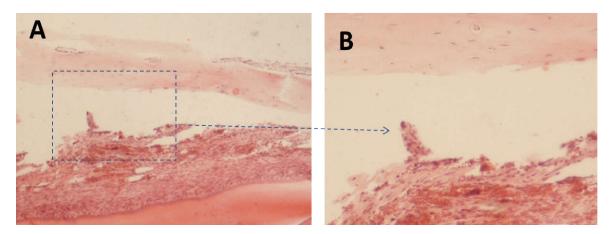
Fig(2). Intraoperative photograph, A: group 1, B: group 2



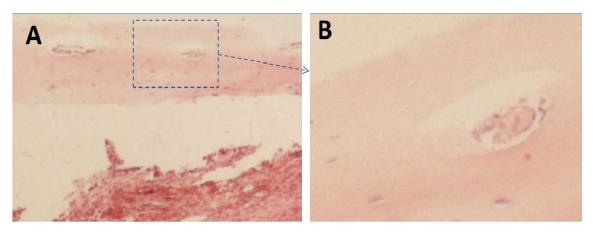
Fig(3). Histologic analyses of 8 weeks biopsy sample of group 1: A: (H&E staining, X 40), B: (H&E staining, X 100)



 $Fig(4).\ Histologic\ analyses\ of\ 8\ weeks\ biopsy\ sample\ of\ group\ 1:\ A:\ (H\&E\ staining,\ X\ 100),\ B:\ (H\&E\ staining,\ X\ 400)$ 



Fig(5). Histologic analyses of 8 weeks biopsy sample of group 2: A: (H&E staining, X 40), B: (H&E staining, X 100)



Fig(6). Histologic analyses of 8 weeks biopsy sample of group 1: A: (H&E staining, X 100), B: (H&E staining, X 400

#### **DISCUSSION**

The purpose of this study was to evaluate the role of internal layer of periosteum in a new bone formation in the dynamic periosteal distraction. The results of this study showed that using non perforated plate hinders the bone formation. The histological evaluation showed that the bone formation was limited to a thin layer of a newly under the titanium mesh, on the other hand, a wider area of a new bone formation have been detected under perforated plate which confirm the role of internal layer in this process. These results are in contrast with the results of Zakaria et al, they evaluated the gradual elevation of the barrier membrane which is initially placed on the bone surface and they concluded that gradually increasing the space over the bone could produce new bone efficiently without sharing of periosteum (Zakaria et al., 2012). The same results were confirmed by saulacic et al, they reported that elevation of periosteum with collagen membrane covering the perforated titanium plate, produces more new bone compared to the elevation with the perforated titanium plate alone, which clarify the benefit of using a barrier membrane over distraction device (Saulacic et al., 2012). However, in the previous reports, most of the devices for the dynamic PDO technique had perforated meshes without standardization of their number or size (Zakaria et al., 2012; Sencimen et al., 2007; Kessler et al., 2007). In agreement with study Yamauchi K et al confirmed that in the dynamic periosteal distraction, it seems to be important to have sufficient communication between the periosteum and the underside of the device to encourage new bone formation (Yamauchi et al., 2013).

#### Conclusion

The results of the present study indicated that the internal layer of periosteum has important role in bone formation in the the dynamic periosteal distraction.

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