THE ROLE OF THE INTERNAL LAYER OF PERIOSTEUM IN BONE FORMATION IN PERIOSTEAL DISTRACTION OSTEOGENESIS: AN EXPERIMENTAL STUDY IN THE RABBITS

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ABSTRACT

The purpose of this study is to investigate the role of the internal layer of periosteum in bone formation in periosteal distraction osteogenesis. A custom-made device was placed between the periosteum and lateral surface of the mandible in 10 adult rabbits. 5 Rabbits were sacrificed after 4 weeks and 5 after 8 weeks. Specimens were fixed, decalcified, and stained with hematoxylin and eosin. Histologic examination was performed on all specimens. The histological analysis of specimens extracted from the periosteal chambers at 8 weeks showed small areas of newly formed bone covered by a lining of osteoblasts, followed by a zone of loose granulation tissue in remaining spaces. On the other hand, some woven bone formation was encountered under the bone chambers of distracted devices. Both of periosteum and basal bone are responsible for bone formation in periosteal distraction osteogenesis and the interaction between them are imperative to produce high quality bone.

INTRODUCTION

By publishing the article “Sur le Development et la CruedesOs des Animaux” in 1742, Duhamel could be considered the first investigator to study the osteogenic potential of periosteum (BilkayU, et al. 2008). Later, the osteogenic/chondrogenic capacity of periosteum, and related mechanisms have been confirmed through a number of studies (Rauch F, et al. 2006, Seeman, E.2003, Emans PJ, et al.2005, Estrada, J.I., et al.2006). Previous studies demonstrated that the immediately elevated periosteum of adult animals did not contribute to the supraosteal bone formation, (Kostopoulos L, et al. 1995, Melcher, A, H, 1971) and the contact between periosteum and bone seems to be essential for the osteogenic capacity of the periosteum (Canalis RF, et al.1985). Recently, osteogenesis by periosteal distraction by gradually lifting the periosteum using different devices without corticotomy or bone augmentation has been suggested (OdaiF, et al.2009, Schmidt BL, et al.2002, Tudor C, et al.2010, Lethaus B, et al.2010) 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 (Kostopoulos L, et al.1995, Schmidt BL, et al.2002). On the other hand, results of other study showed that the newly formed bone in periosteal distraction was mainly produced from the basal bone, not from the periosteum (ZakariaO, et al.2012). Since the results of several investigators about the main responsible of osteogenesis in PDO are conflicting, the purpose of this study is to investigate the role of the internal layer of periosteum in bone formation in periosteal distraction osteogenesis.

MATERIALS AND METHODS

Experimental animals

Ten adult White male rabbits with a mean weight of 2.6 ± 0.39 kg were used as the animal model. Experimental protocols were approved by University of Al Andalus university Committee of Animal Research. A custom-made device was placed between the periosteum and lateral surface of the mandible in 10 rabbits. 5 Rabbits were sacrificed after 4 weeks and 5 after 8 weeks.

Distraction device

The periosteal distraction device was a custom-made device of titanium (height 4 mm, width 4 mm, length 12 mm). The device has two contrary chambers, one of them faces the internal layer of periosteum (periosteal chamber) and the other one faces the surface of bone (bone chamber), the device could...
be rigidly fixed to the lateral aspect of the mandibular ramus using 1-3 mm titanium screws. Fig. (1)

Figure 1 the Custom-Made Periosteal Distraction Device, A: Periosteal Chamber, B Bone Chamber

Surgical procedures

All surgical procedures were performed under general anesthesia with a combination of 35 mg/kg intra muscular 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 was made in the skin 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. The distracted device was rigidly fixed with two titanium screws to the bone. The wound was closed in layers, using 4-0 Vicryl sutures. Postoperative analgesics included ketorolac (0.5 mg/kg by mouth) and buprenorphine (0.3 mg intramuscular). Fig. (2)

Figure 2 Intraoperative Photograph, A: Submandibular Dissection, B: Rigid Fixation of The Periosteal Distraction Device To The Lateral Aspect of The Ramus

Specimen preparation

After healing periods of 4 and 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

After 4 weeks

Histological analysis of specimens extracted from the periosteal chambers of distracted devices showed an increase in periosteal proliferation, high vascularity and collagen fibers. Areas of early bone formation surrounded by osteoblasts, and an increase in the number of osteocytes per unit area were detected Fig (3). On the other hand, periosteal proliferation and new blood vessels have been detected under the bone chambers of distracted devices Fig (4).

Figure 3 Histologic analyses of a 4 weeks biopsy sample from periosteal chamber: periosteal proliferation, newly formed bone surrounded by osteoblasts. (H&E staining, X 40)

Figure 4 Histologic analyses of a 4 weeks biopsy sample from bone chamber: periosteal proliferation, new blood vessels. (H&E staining, X 100)

After 8 weeks

Histological analysis of specimens extracted from the periosteal chambers showed small areas of newly formed bone. The newly-formed bone was covered by a lining of osteoblasts, followed by a zone of loose granulation tissue in the remaining space between the trabecular bone. Fig (5).

Figure 5 Histologic analyses of an 8 weeks biopsy sample from periosteal chamber: Newly formed, dense bone tissue, Increase in thickness of new bone below the periosteum. (H&E staining, X 40)

Figure 6 Histologic analyses of an 8 weeks biopsy sample from bone chamber: immature osteoblasts at the margin of newly formed bone surrounded by collagen fibers (H&E staining, X 100)
periosteal proliferation and some woven bone formation were encountered under the bone chambers of the distracted devices. The new bone was surrounded with immature osteoblasts and connective tissue. Fig (6).

**DISCUSSION**

In this study we used a custom-made device to investigate the role of the internal layer of periosteum and the basal bone in bone formation in Periosteal distraction osteogenesis. The device have two contrary chambers to study the role of internal layer of periosteum and the basal bone independently.

Preservation of the periosteum is important for bone formation (YasuiN, et al 1991) because it contains stem cells as osteoprogenitors (Nijweide PJ, et al 1986) and plays central roles in skeletal development and remodeling (Ilizarov GA. 1989 ). (Kostopoulos et al, 1995) cleared that the outer surface of periosteum, exhibits significantly more bone fill capacity than the inner surface of the elevated and repositioned periosteum in addition, the new bone which formed by periosteum becomes resorbed with time.

The histological analysis of this study showed the both the basal bone an periosteum are responsible for bone formation. Weng et al (WengD, et al. 2000) investigated the role of periosteum in de novo bone formation by covering a custom-made hemispherical titanium mesh with ePTFE membrane to prevent connective tissues from invading the formed space. On the control side the mesh was left uncovered. New bone was found on the outside of the existing bone with a new periosteal layer on top. They concluded that periosteum does not seem to contribute to the formation of new bone tissue. The same findings using titanium cylinders (6.2 mm height) in the rabbit skull were also reported by Lundgren et al (Lundgren A.K., et al.2000 ). Yamada et al, (Yamada Y,et al,2003) evaluated the effects of occlusiveness of a titanium cap with or without small holes on bone generation. The results of their study showed that the amount of newly generated bone is significantly increased inside non-perforated cap in 3-month specimens. In contrary to these results.

Takiguchi et al, (Takiguchi. S et al, 2009) suggested that the periosteum plays an important role in promoting new bone formation and the removal of periosteum delays this process, in their study new bone formation was observed centering on the calvarial bone from 2 weeks after the operation when the periosteum had preserved, on the other hand it was not observed until 6 weeks when the periosteum removed. Also Tudor et al, (Tudor C et al, 2010) postulated that the perforation of the meshes is an imperative to enable and guarantee sufficient communication between the periosteum and the underlying space. A solid mesh would prevent, or at least reduce, the healing capacity in the newly created space. Recently the study of Dziewiecki et al, (Dziewiecki D. et al, 2016) showed that osteogenesis mainly occurred at the interphase between the stretched periosteum and the devices and only minimal newly formed bone was detected inside the devices , they stated that the insufficient permeability of the devices led to insufficient formation of a stable blood clot under the device. They concluded that periosteal elevation can produce new bone formation which derives from the periosteum and the underlying bone.

In our study the bone formation in periosteal chambers was limited to small areas. The role of the mesh-perforations is still a matter of debate. In the previous reports, most of the devices for the dynamic PDO technique had perforated meshes without standardization of their number or size (Zakaria O,ET AL.2012, Kessler P, et al.2007). In the dynamic periosteal distraction, it seems to be important to have sufficient communication between the periosteum and the underside of the device with appropriate mechanical strength against the overlying soft tissue to encourage new bone formation (Yamauchi K,et al.2013).On the other hand, it has been 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 N, et al.2013). This is in accordance with Zakaria et al study (ZakariaO,et al,2012 ). In their study the histological finding demonstrated that newly formed bone originated mainly from the progenitor cells of blood vessels and from osteoblasts which were provided from the basal bone through the perforated bone holes. Their results about the role of periosteum have been confirmed in their following study when 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 (ZakariaO, et al.2012). Minimal newly bone formation was observed under the bone chamber in our study was thus indicate the necessity of interaction between the basal bone and internal layer of periosteum to produce high quality bone.

**CONCLUSION**

within the limits of the present study, both of periosteum and basal bone are responsible for bone formation in periosteal distraction osteogenesis is and the interaction between them are imperative to produce high quality bone.

**References**


