THE ROLE OF STATIC PERIOSTEAL DISTRACTION WITH PLASMA RICH IN GROWTH FACTORS (PRGFS) IN BONE FORMATION: AN EXPERIMENTAL STUDY IN THE RABBITS

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Osteogenesis by "periosteal distraction (PDO) without corticotomy has been suggested as a new technique for bone augmentation. Various methods have been used to enhance bone healing in the distracted area. This study was aimed to evaluate the role of static periosteal distraction with plasma rich in growth factors (PRGFs) in a new bone formation. Titanium mesh was placed between the periosteum and lateral surface of the mandible in 10 adult rabbits. The space between the surface of bone and titanium mesh was filled with PRGFS soaked onto a pre-cut absorbable collagen sponge in 5 rabbits. The histological evaluation of the specimens extracted from the test group showed athick newly woven bone the on the lateral side of the mandible under the titanium mesh and the new bone is separated from the original bone by a thin layer of connective tissue. The results of the present study indicated that application of plasma rich in growth factors (PRGFs) into the site of periosteal distraction improves the bone formation.

Abstract

The purpose: This study was aimed to evaluate the role of static periosteal distraction with plasma rich in growth factors (PRGFs) in a new bone formation.

Materials and methods:

Titanium mesh was placed between the periosteum and the lateral surface of the mandible in 10 adult rabbits. The periosteum was distracted 3-4 mm apart from the surface of the bone. The space between the surface of bone and titanium mesh was filled with PRGFS soaked onto a pre-cut absorbable collagen sponge in 5 rabbits (test group) and the others without PRGFS (control group).Rabbits were sacrificed after 8 weeks. Specimens were fixed, decalcified, and stained with hematoxylin and eosin.

Results: The histological evaluation of the specimens extracted from the control group showed a newly thin layer of osteoid bone under the titanium mesh. The new bone is

separated from the original bone by a thick layer of a connective tissue. On the other hand, the histological evaluation in the test group showed athick newly woven bone on the lateral side of the mandible under the titanium mesh and the new bone is separated from the original bone by a thin layer of connective tissue**. Conclusion:** The results of the present study indicated that application of plasma rich in growth factors (PRGFs) into the site of periosteal distraction improves the bone formation.

Keywords: bone formation, osteogenesis, periosteal distraction, plasma rich in growth factors (PRGFs).

INTRODUCTION:

Recently, osteogenesis by "periosteal distraction (PDO) without corticotomy has been suggested as a new technique for bone augmentation(*Lundgren et al.2000; Schmidt et al.2002;Yamauchi et al.2008;Weng et al.2000*).Although the application of this technique results in de novo bone formation, the previous studies presented evident heterogeneity with respect to the surgical technique, the used device, the distraction rate(*Al Nasharet al.2016;Al Nasharet al.2016*). Additionally, the quality and the quantity of the newly formed bone are less than ideal compared with that produced by DO(*Sencimenet al.2007;Altuget al.2011*).New techniques have been suggested to enhance the bone formation in distracted space and led to positive results(*Casap et al.2008;Pripatnanont et al.2015*).Plasma Rich in Growth Factors (PRGFs) is derived from autologous blood by sequestering and concentrating the platelets by centrifugation (*Anitua 1999; Al Nashar&Yakoob. 2015;Al Nasharet al.2016*).It is advocated the platelet concentration can enhance oral wound healing by releasing abundant growth factors, including platelet-derived growth factor (PDGF), transforming growth factor- (TGF-), insulin-like growth factor (IGF) and epidermal growth factor (*Anitua,2007*). The purpose of this study is to evaluate the role of static periosteal distraction with plasma rich in growth factors (PRGFs) in a new bone formation.

MATERIALS AND METHODS:

Experimental animals:

Ten adult male rabbits with a mean weight of 2.3 ± 0.29 kg were used as the animal model. Experimental protocols were approved by University of Al Andalus university Committee of Animal Research.

Preparation of PRGFs:

Before surgery and the administration of local anesthesia, 10 ml of peripheral blood was drawn. The blood was deposited in laboratory glass tubes pre-treated with 3.8% trisodium citrate. The tubes were centrifuged at 270 rpm at room temperature for 7 min in a centrifuge unit specifically designed for use with this technique (PRGF System; BTI Biotechnology Institute, Vitoria-Gasteiz, Alava, Spain). This allows the separation of blood into distinct layers: a cellular layer at the bottom, PRP in the middle, and platelet-poor plasma at the top. The cellular components (mostly red blood cells and a thin layer of white blood cells) remain at the bottom of the tube, above which is the plasma component consisting of PRGFs and finally a layer of plasma poor in growth factors. The middle layer was collected and stored in a sterile glass container until use. Leukocytes were not collected in this preparation. At the time of the application, approximately 50 µl of 10% CaCl2 solution was added per 1 ml of PRGF concentrate to enable clot formation. A platelet cell count was done before and after centrifugation.

Surgical procedure:

All surgical procedures were performed under general anaesthesia with a combination of 35 mg/kg intramuscular ketamine and 5 mg/kg subcutaneous xylazine. Local anaesthesia, 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 periosteum was distracted with a titanium mesh 0.1 mm in thickness and was cut to a size of approximately 12 ×15 mm and adjusted to distract the periosteum 4 mm apart the surface of bone. In the test group, the space between the surface of bone and titanium mesh was filled with PRGFS soaked onto a pre-cut absorbable collagen. 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).

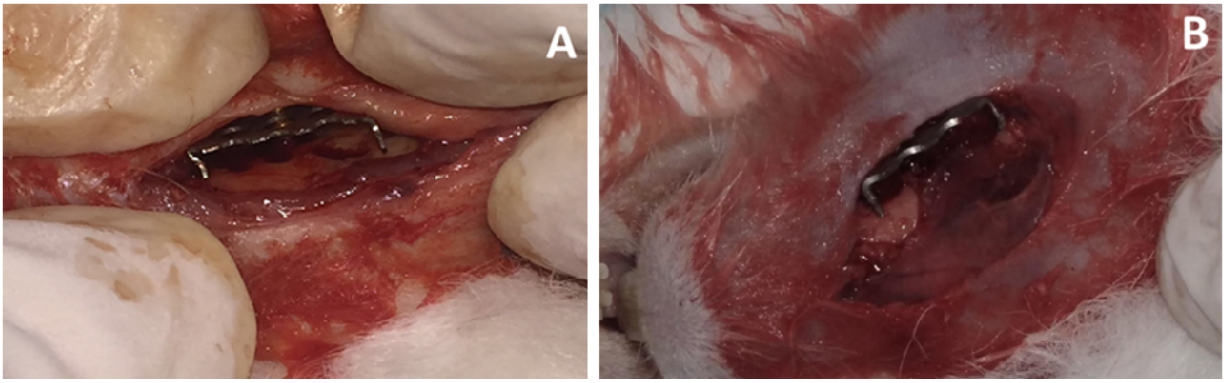

Figure1. Intraoperative photograph, a: control group, B: test group

Specimen preparation:

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.

Control group:

Histological evaluation showed a thin layer of a newly osteoid bone with high vascularity on the lateral side of the mandible under the titanium mesh, The bone was covered by a lining of osteoblasts. The new bone is separated from the original bone by athickconnective tissue which is rich incollagen fibers, cells, new blood vessels and a layer of periosteal proliferation.

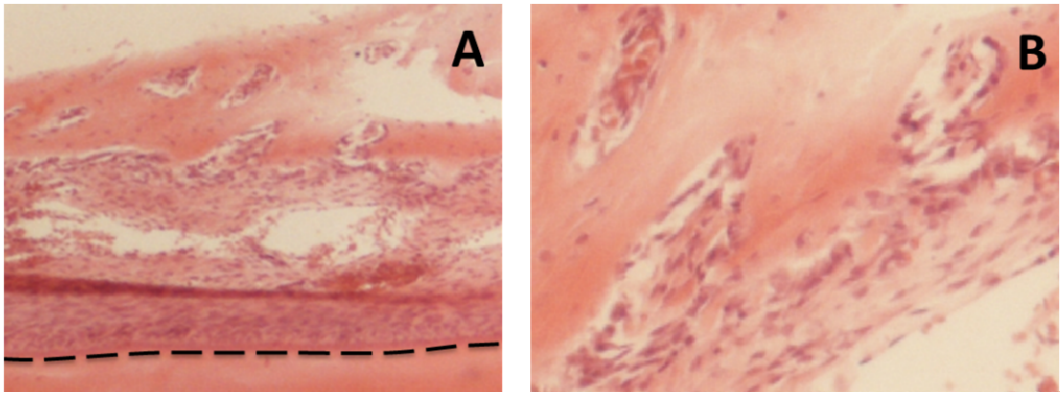

Figure 1. Histologic analyses of 8 weeks biopsy sample of control group: A: (H&E staining, X 40), B: (H&E staining, X 100),

Test group:

Histological evaluation showed a thick newly woven bone on the lateral side of the mandible under the titanium mesh. The bone also was 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.

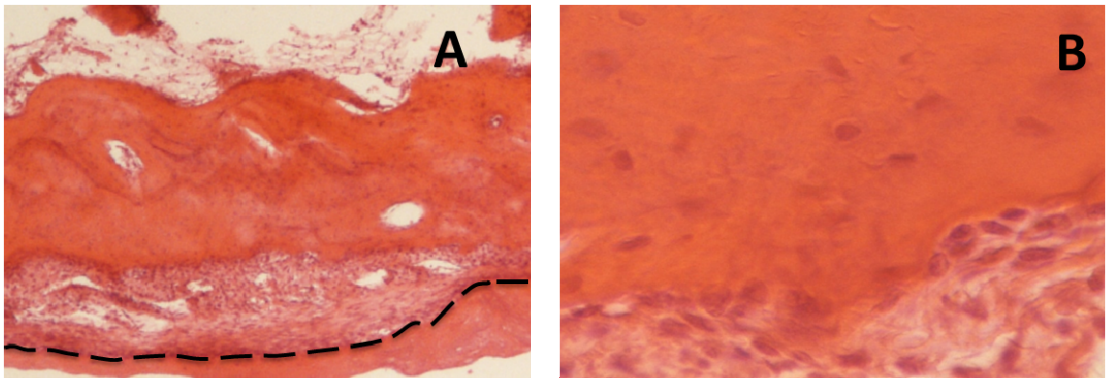

Figure 2. Histologic analyses of 8 weeks biopsy sample of test group, A: (H&E staining, X 40), B: (H&E staining, X 100),

Discussion:

Recently, it was reported that periosteal distraction can induce a new bone formation. 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 (*Kessler et al.2007;Zakariaet al.2012;Takiguchiet al.2009*) .However, Sencimen et al.(*Sencimen et al.2007*) reported that insufficient mature bone formation was observed in periosteal

distraction alone, although fairly a dense bone formation was observed in DO.Altug et al.(*Altug et al.2011*) also used the mandible of the rabbit as a model to perform periosteal distraction. They reported an abundance of adipose tissue within the newly formed bone. In our study, the histological evaluation of the specimens extracted from the control group showed a newly thin layer of osteoid bone under the titanium mesh. The new bone is separated from the original bone by a thick layer of connective tissue. On the other hand, the histological evaluation in the test group showed a thick newly woven bone the on the lateral side of the mandible under the titanium mesh and the new bone is separated from the original bone by a thin layer of connective tissue. Sato et al.(*Sato et al.2010*)investigated whether the administration of mesenchymal stem cells into the gap between bone surface and periosteum promotes bone formation, the experimental group in their study showed significantly increased volume, height, bone mineral density, and bonemineral content. Casapa et al. (*Casapa et al.2008*) investigated the intracallus administration of vascular endothelial growth factor (VEGF) into the distracted area, they concluded that VEGF has a positive effect on osteogenesis. Pripatnanontet al (*Pripatnanont et al .2015*) evaluated the effect of a modified Hyrax device and platelet-rich fibrin (PRF) on osteogenic periosteal distraction, they confirmed that a greater bone maturation was achieved with the addition of PRF. Suer et al (*Suer et al .2014*)investigated the effects of the hyperbaric oxygen HBO on de novo bone formation during periosteal distraction, their results indicated that periosteal distraction with HBO could be used to increase the quality and quantity of the bone newly formed by POD. Whereas, the local application of simvastatin to the distractionzone made no significant contribution to the new bone formation (*Kahraman et al.2015*)

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Conclusion:

The results of the present study indicated that application of plasma rich in growth factors (PRGFs) into the site of periosteal distraction improve the bone formation.

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