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THE EFFECT OF NANOCRYSTALLINE HYDROXYAPATITE -PARATHYROID HORMONE MIXTURE ON BONE DEFECT HEALING: EXPERIMENTAL STUDY IN DOGS

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ABSTRACT

Objectives: The study aimed to evaluate the histological effect of nanocrystalline hydroxyapatite mixed with parathyroid hormone on the healing of mandibular bone defect.

Material & Methods: The present study was conducted on 18 skeletally mature mongrel male dogs. The animals were randomly assigned (1:1) to one of the two groups (9 animals each) according to the treatment method: Study group where animal received PTH- Nanobone® mixture in a created osseous defects and control group where animal received Nanobone® solely within the defect. Three animals from each group were sacrificed at 3, 6 and 12 weeks postoperative. All groups were analyzed histological for the closure of the created osseous defect, the characteristics of the developed connective tissue, the nature of the formed osteoid matrix, the presence of acute or chronic inflammatory cells, and the type and progression of the healing process.

Results: The histological examination showed significant difference between the 2 groups with better bone healing at Study group (PTH- Nanobone group). As the histological section Study group (PTH- Nanobone group) showed newly formed thin bone bridges of woven bone at 3 weeks postoperative while at 6weeks bone bridges of woven bone became thicker. At 12 weeks follow-up, both groups showed signs of healing although Study group (PTH- Nanobone group) had a more lamellar and well organized bone is formed with newly haversian systems with wide osteons become evident.

Conclusion: The results suggest that nanocrystalline hydroxyapatite mixed with parathyroid hormone encouraged the bone healing in critical-size calvarial defects

KEY WORDS: Parathyroid hormone, Nanocrystalline Hydroxyapatite, bone healing, bone defect, bone regeneration.

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INTRODUCTION

Bone loss is considered morbid ailment that affect the quality of patient's life mostly occurred due to existence of tumors or cysts, trauma, or infectious disorders [1, 2]. The deformity caused by bone loss may cause functional, aesthetic, or structural difficulties within the jaw, impairing mastication and speaking. Although bone tissues has favorable capacities of regeneration, these repairing capacity had limited effect when the bone loss is massive and healing is a challenging process. As a result of all these issues, reconstructive surgery has become increasingly popular. Filling such defects is necessary to preserve teeth and ensure appropriate bone support for subsequent restoration with either a partial, fixed prosthesis, or dental implants.[1-3]

Various materials, such as autogenous, allogeneic, xenogeneic, and alloplastic materials, are available for reconstruction of such defects. Although, autogenous bone grafts are still regarded as the gold standard, their disadvantages include higher rate of postoperative morbidity in the donor site as prolonged pain and nerve injury [3,4]

Allografts are another option that comes in a variety of forms, including demineralized bone allograft (DFDBA), freeze-dried bone allograft (FDBA), and fresh frozen bone (FFB). It is necessary to create some stringent rules for their safe use, such as adequate donor selection, bone harvesting, processing, and storage techniques. However, limited availability, the risk of antigenicity, immunologic reactivity, and cross infection all pose challenges in the selection of these materials for their use in maxillofacial reconstruction. Moreover, human recipients are biocompatible with xenografts, which come from animal species. They are more plentiful and come in a variety of sizes, and they have an osteoconductive action. Disease transmission and immunogenicity are still a major problem when dealing with their use [3, 5,6].

Synthetic bone grafting materials have been created to address the issues associated with the use of the previously mentioned grafts. Bioactive glasses, tricalcium phosphates (TCP), and hydroxyapatite (HA) are some of the most utilized alloplastic materials. They have a lot of advantages over natural materials, including higher availability, no risk of disease transmission, and a lower level of antigenicity. They can be produced in a variety of ways, each with its own set of physicochemical features. They are mostly osteoconductive and have no influence on osteogenesis or osteoinduction.[6-9]

Nanomaterials have recently become popular in the medical field. When employed for bone regeneration operations, they have considerable surface, quantum, and size effects, which contribute to superior performance than standard materials.[10]

The nano-crystalline hydroxyapatite (Nano-bone®) bone transplant is one of these materials. It's encased in a silica gel matrix that is permeable. Nanobone® has the benefit of being a bioresorb-able and osteoconductive material. Rapid repair of critical-size defects was shown in animal trials and human applications because this substance attaches to bone and encourages bone healing by increasing osteoblastic activity.[10-13]

Although the relatively satisfactory results of the synthetic bone grafts, there are associated drawbacks and limitations to their use and availability, and even controversial reports about their efficacy and cost-effectiveness. Furthermore, there are no bone substitutes available that have superior or even the same biological properties compared with autogenous bone graft. Therefore, there is a necessity to develop adjuncts substances improve the biological outcome of synthetic bone graft in an effort to overcome these limitations. The FDA approved techniques have led to wide progress in exploration of bioactive material to improve the bone regeneration. [3, 9, 14].

Parathyroid hormone (PTH) is a protein hormone that is secreted by parathyroid gland. It is available at the market as recombinant PTH and available to treat bone disorders such as osteoporosis. PTH

stimulates both cortical and cancellous bone regeneration, increases bone volume, and improves mechanical bone strength and density with a safe profile, according to several animal and clinical investigations. A vast number of animal studies have been conducted to prove the effectiveness of this therapy option. PTH appears to be effective in the treatment of fractures because it speeds up the healing and regeneration processes. [15-19]

Although there was positive effect of PTH, continuous elevation of PTH serum level may lead to catabolic effect in bone. That direct a researchers to provide PTH to the local site to induce the optimum PTH anabolic action and preserve the PTH bioactivity. [18,19]

There are few studies evaluating the effect of parathyroid hormone local application as a therapeutic agent for bone healing. This study was conducted to evaluate the histological effect of nanocrystalline hydroxyl apatite mixed with parathyroid hormone on the healing of mandibular bone defect.

MATERIAL & METHODS

Animal & Experimental designs

The present study was conducted on 18 skeletally mature mongrel male dogs. Each dog was given a complete clinical, physical and radiographic examination to exclude any evidence of systemic, orthopedic and neurologic diseases. Dogs included in the study were with a 13± 1.3 kg body weight and average age 15±2.3 months. The animals were housed and quarantined individually in separate cages at the department of Physiology, Faculty of Medicine, Alexandria University for one week to become acclimatized to the housing and diet.

The animals were randomly assigned (1:1) to one of the two groups (9 animals each) according to the treatment method: Study group where animal received PTH- Nanobone® mixture in a created osseous defects and control group where animal received Nanobone® solely within the defect. The randomization was simple and carried out via predefined computer-generated random codes enclosed in sealed opaque envelope.

All animal experiments and surgical procedures were conducted according to the laws of animal protection and welfare and all the study procedures were approved by the ethical committee of Fayoum University. The animals were fastened for 12 hrs. prior to surgery and allow free access to drinking water till time of anesthesia.

Agent and Biomaterials

The created osseous defect was filled with Nanobone® (Nanobone®, ARTOSS GmbH, Rostock, Germany). The Nanobone is a silica gel matrix with nanocrystalline HA incorporated in it. It comes in two sizes: fine (0.6mm x 2mm) and rough (1mm x 2mm). At temperatures below 200°C, all Nanobone® technology products are made in a sol-gel process. Because the material is not sintered due to the low temperatures, its surface is very porous, with pores ranging in size from nanometers to micrometres. The blood's autologous proteins enter the nonporous and coat the entire inner surface. In the study group, the graft material was mixed with PTH (PTH (1-34), Kaneka Eurogenetic, Seraing, Belgium). The amount of the solution used for mixing with the graft was corresponding to 1 ml.(Fig. 1)

Fig. (1): Photograph showing bottle of PTH (1-34)

Surgical Procedures

According to a veterinarian report, all the dogs were in good health. Throughout the duration of the study, all dogs were fed the same balanced diet of milk, broth, and meat. Just before the operation, each animal was given a single dosage of antibiotics intravenously in the form of (Ampicillin 25 mg/kg body weight). In an animal theatre, all surgical procedures were carried out under general anesthesia and under rigorous sterile circumstances. Each animal was given a general anesthetic via intravenous injection of (thiopentone sodium 5%), the dose of which was calculated at 30 mg/kg/ body weight.

A 5cm gingival incision was made on the buccal side in the premolar region of the left side of the body of the mandible, then a mucoperiosteal flap was elevated. An Osseous defect of 10mm depth and 10mm width was established with the use of a trephine bur on an electric motor under copious saline irrigation. For the study group, PTH- Nanobone® was placed into the created defect, while for the control group, Nanobone® was packed only into the osseous defect. The flap was then sutured with 3/0 chromic cat gut. (Fig. 2)

After surgery, the animals were transferred to a clean cage and kept under observation to assess the presence or absence of infection, wound dehiscence, or graft rejection. They were kept on a soft diet consisting of bread, milk, and broth for the first four weeks after surgery. All dogs received intramuscular injections of (25-50mg/kg/dog of ampicillin) every 24 hours for five days. All the dogs were given the same anti-inflammatory and analgesic medicine (diclofenac potassium 25mg/day) twice daily for three days.

The animals were clinically examined for the presence or absence of infection or any emerging problems, as well as the general condition and healing of the surgical sites, on the first few postoperative days and then weekly until the end of the follow-up period.

Animal Euthanasia

Animals were sacrificed at 3, 6 and 12 weeks postoperative. (3 animals from each group at each follow-up interval), using a combination of xylazine (20mg/ kg) and ketamine (50 mg/kg) administrated intramuscular followed by with an overdose of sodium pentobarbital (60 mg/ml).

Histological preparation

Upon completion of the experimental periods for each group, the animals were euthanized, and the area of the original surgical defect and the surrounding tissues were removed En bloc and fixed with 10% buffered formalin for 48 hours. The specimens were then decalcified in 10% EDTA (PH: 7.4) solution for 3 weeks. The solution changed every week. Upon complete decalcification the specimens cut transversely next to the hole and embedded in paraffin according to standard histological procedures. Five micrometer thick serial sections were cut, stained with hematoxylin and eosin stain. The sections were observed under a light microscope (Olympus BX61, Hamburg, Germany) connected to a high-resolution digital camera (Olympus, E330, Imaging Corp), whereby digital images were obtained for each section in the two time intervals by a single unbiased oral pathologist.

The best sections (which were given codes) were used for histological evaluation were the closure of the created osseous defect, the characteristics of the developed connective tissue, the nature of the formed osteoid matrix, the presence of acute or chronic inflammatory cells, and the type and progression of the healing process were evaluated. The Pathologist's observations were tabulated and then the codes were revealed by the authors.

RESULTS

Surgery and postoperative period

Surgery and anesthesia were uneventful. No signs of discomfort or unusual reactions were seen in animals during the operation. During the

healing period, no deep or superficial infection were recorded. All animals showed normal food and water intake 10 days after surgery. Upon retrieval of the bone and surrounding tissues all sites revealed undisturbed healing. There were no signs of inflammatory or foreign body reaction was visible at any time interval.

Histological evaluation

After 3 weeks follow-up interval, the examined Histological sections stained with (H&E) of the Control group (Nanobone group) showed the old mandibular bone Indicated with letter D, only granulation tissue (red arrow) that was composed of newly formed capillaries & fibrous connective and inflammatory cells were detected, (green arrow) indicating some residual necrotic bone from the operation site. while in Study group (PTH- Nanobone group), the examined Histological sections stained with (H&E) Remnant of material surrounded by newly formed thin bone bridges of woven bone (green arrow) with wide interstitial tissue spaces between the newly formed bone and the remnants of the material, thick fibrous connective tissue at the periphery (blue arrows), osteoid bone formation (red arrows) (Fig. 3)

After 6 weeks follow-up interval, the examined Histological sections stained with (H&E) of the Control group (Nanobone group) showed Bone trabeculation started to appear but surrounded with granulation tissue (green arrow), at the periphery post osteoclastic activity appeared in the form of scalloping indicating bone remodeling activity as a step in healing process (red arrow) . On the other hand, Study group (PTH- Nanobone group) showed newly formed bone bridges of woven bone became more thicker showing different degrees of basophilia (red arrow) with wide cavities between the bone trabeculae, scalloping shows some degree of osteoclastic activity indicating bone remodeling potentialities, green arrows showing apparently small areas of fibrous tissue (Fig.4)

After 12 weeks follow-up interval, the examined Histological sections stained with (H&E) in the Control group (Nanobone group) showing that Still woven bone resident showing many reversal lines, areas of bone remodeling (red arrow) and fibrous connective tissue (green arrow) with inflammatory cells subsides. However, Study group (PTH- Nanobone group) Histological sections stained with (H&E) showed union or fusion of the newly formed bone and the old became evident with osteoid bone (red arrows), in the middle areas of fibrous tissue (blue arrows) can be detected, remodeling appeared as more lamellar and well organized bone is formed with newly haversian systems (green arrow) with wide osteons become evident as a process of lamellar bone formation (Fig. 5)

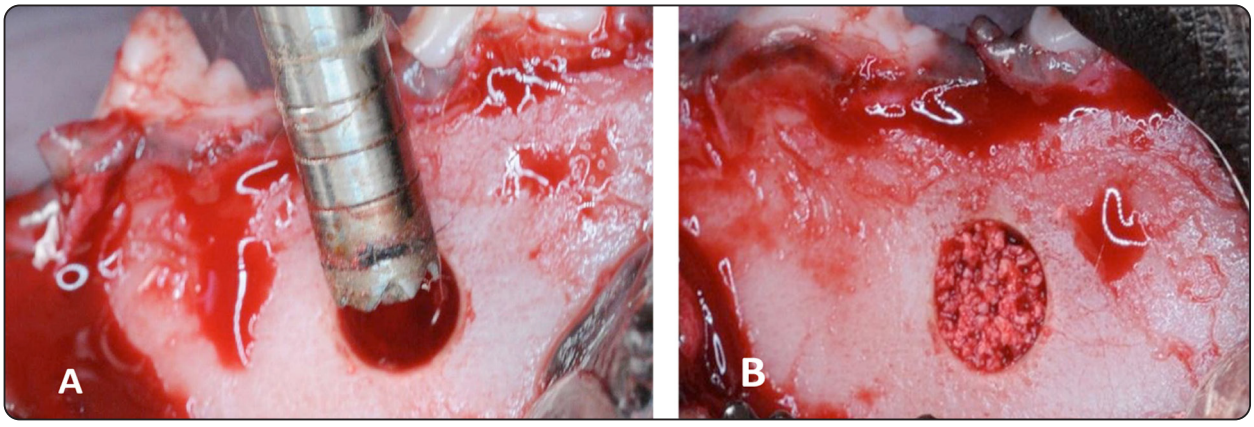

Fig. (2) Photograph showing the surgical procedures A- creation of surgical defect, B- packing of Nanobone in the surgical defect

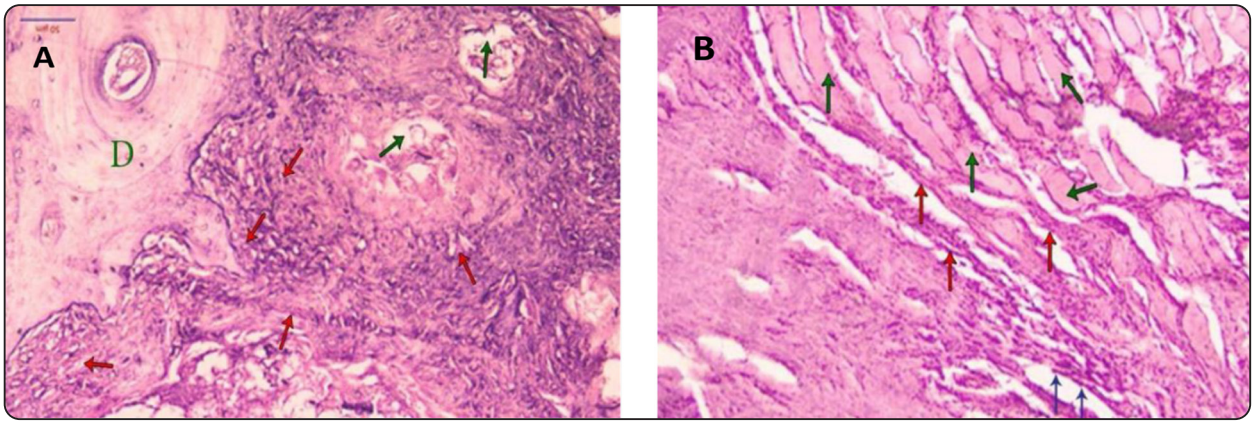

Fig. (3): HE staining of histological sections 3 weeks postoperative A Control group (Nanobone group) b- Study group (PTH-Nanobone group)

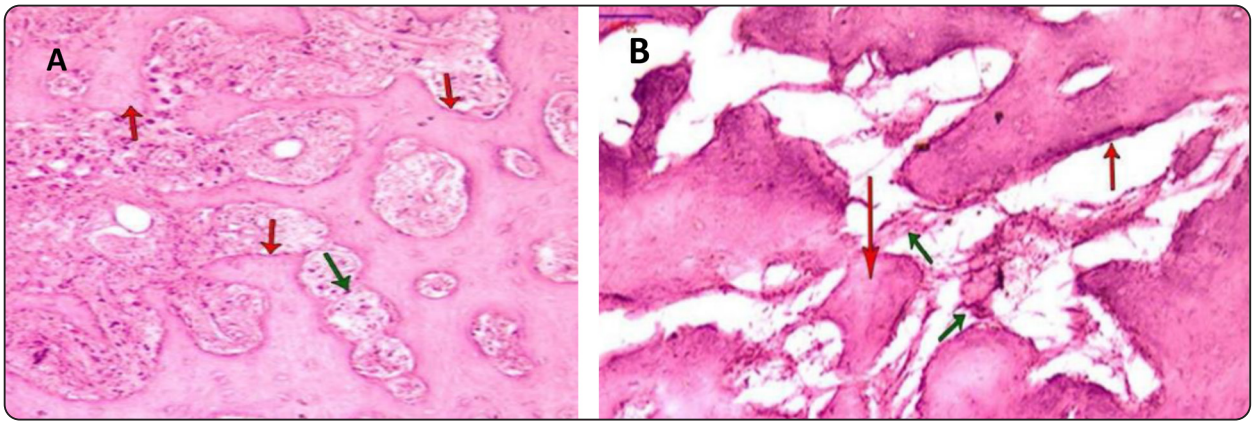

Fig. (4): HE staining of histological sections 6 weeks postoperative A Control group (Nanobone group) b- Study group (PTH-Nanobone group)

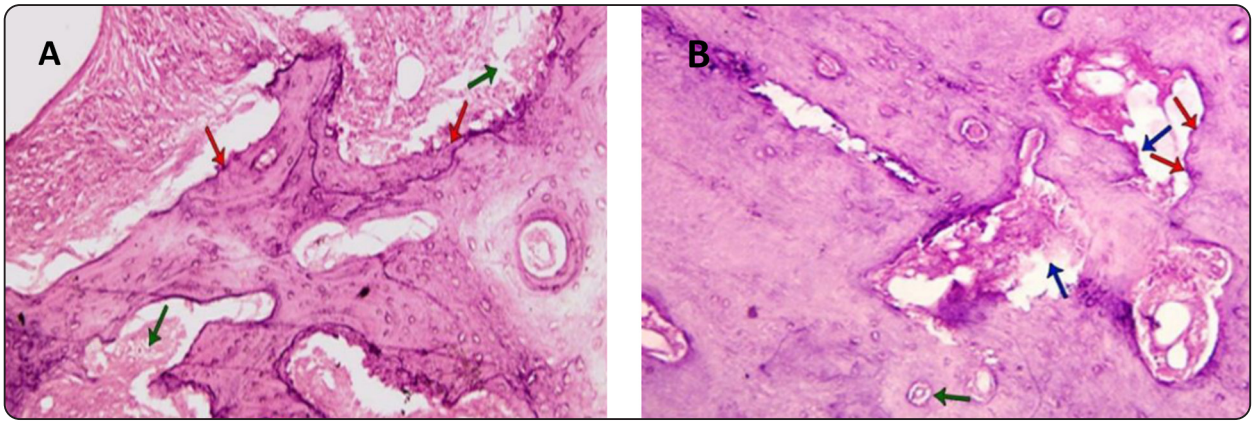

Fig. (5): HE staining of histological sections 12 weeks postoperative A Control group (Nanobone group) b- Study group (PTH-Nanobone group)

DISCUSSION

The current approaches to improve bone critical size defect regeneration include application of autografts, allografts, bioactive factors, biocompatible implants and mesenchymal stem cells. However, those procedures face significant limitations due to insufficient blood supply, potential disease transmission, less ability of functional engraftment, or immunorejection. Additionally, vascularization of 3-dimensional scaffolds for tissue regeneration remains a challenge. Thus, FDA approved use of pharmacological agent to improve bone repair and healing. The use of proper bioactive material remains a great demand and clinical challenge in oral and maxillofacial surgery. [1-4]

The present study was conducted to evaluate the histological effect of nanocrystalline hydroxyl apatite mixed with parathyroid hormone on the healing of mandibular bone defect. The PTH hormone is a peptide hormone secreted from parathyroid gland. It is a key factor in regulating the balance of calcium and phosphorus in the blood through action on bone and kidney as well as calcium absorption from intestine [20, 21]. The present study used PTH (1-34). The teriparatide PTH (1-34) represent the biologically active fragment of PTH. Its administration play a significant role in the promotion of osteoblastic differentiation and had been suggested to stimulate bone formation. The FDA approved the use of PTH has been in the treatment of osteoporosis, and to improve osseointegration of implants. [22-25]

The PTH showed the ability to increase bone mineralization by increasing Ca2+and PO4 absorption and in turn stimulates the bone matrix. The osteoanabolic activity of PTH depends on its ability to increase osteoblast via an increase in osteoblast genesis, a decrease in osteoblast apoptosis, and the differentiation of lining cells into active osteoblasts. Moreover, PTH directly stimulate growth factor receptors that have a critical impact on regulating the osteoblast activity. Additionally, different studies had showed that PTH was associated with anti-catabolic action that improve healing of fractures and boney defects. [26-32]

The results of the present study showed that the bone defect treatment of Study group (PTH-Nanobone group) showed a better healing than Control group (Nanobone group) when all outcomes measurements are considered collectively. After 3 weeks follow-up interval, the examined, Study group (PTH- Nanobone group), showed that remnant of material surrounded by newly formed thin bone bridges of woven bone. While at 6 weeks follow-up interval, the study group showed a more progressive healing as the newly formed bone bridges of woven bone became more thicker showing different degrees of basophilia with wide cavities between the bone trabeculae, scalloping shows some degree of osteoclastic activity indicating bone remodeling potentialities. After 12 weeks follow-up interval, Study group (PTH- Nanobone group) showed union or fusion of the newly formed bone and the old became evident with osteoid bone, in the middle areas of fibrous tissue can be detected, remodeling appeared as more lamellar and well organized bone is formed with newly haversian systems with wide osteons become evident as a process of lamellar bone formation)

That in line with Wodja [33] eta al, who examined the local delivery of parathyroid hormone (PTH) using a thiol-ene hydrogel embedded in a porous poly (propylene fumarate) (PPF) scaffold for bone regeneration applications and concluded to treatment of bone defects with the composite thiol-ene hydrogel-PPF scaffold, delivering either 3 or 10 μg of tethered PTH 1-84, was found to increase bridging of critical size bone defects, whereas treatment with 30 μg of tethered PTH resulted in less bone ingrowth into the defect area.

Moreover, Orth et al [34] who investigate whether systemic application of PTH [1-34] improves the

repair of non-osteoarthritic, focal osteochondral defects and concluded that PTH [1-34] emerges as a promising agent in the treatment of focal osteochondral defects as its systemic administration simultaneously stimulates articular cartilage and subchondral bone repair.

On the other hand, Stancoven [35] et al who investigate potential additive/synergistic effects of exogenous parathyroid hormone and concluded that PTH did not show a significant effect on bone formation.

Moreover, Grossi [36] et al who evaluate the effect of (PTH) administered directly to the implant's surface prior to insertion, using a large translational animal model and concluded that Single local application of different concentrations of PTH on titanium implant's surface did not influence the osseointegration at any time-point evaluation in low-density bone.

The present research provides initial promising results regarding the use of PTH to accelerate bone healing. However, there was limitations that need to be addressed in future studies. The limitations include the number of the animal model on each group and systemic effect of the PTH in local applications and concentration of the drug in the defect. Further studies will be needed to evaluate the long-term outcomes in the bone healing and more studies should be directed toward the safety and tolerability of the drug.

CONCLUSION

The results suggest that nanocrystalline hydroxyapatite mixed with parathyroid hormone encouraged the bone healing in critical-size calvarial defects

Competing interests

The authors declare that they have no competing interests

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