

Histological Evaluation for the Use of β -Tricalcium Phosphate as a Bone Substitute in Accelerating Bone Healing: An Experimental Study on Rabbits

Atalla F Rejab
BDS., MSc., FIBMF. (Lec.)

Bara S Minwah
BDS., MSc., FIBMF. (Lec.)

Yahya A Ameen
BDS., M.Sc. (Asst. Lec.)

Dept. of Oral and Maxillofacial Surgery
College of Dentistry, University of Mosul

Dept. of Oral and Maxillofacial Surgery
College of Dentistry, University of Mosul

Dept. of Oral and Maxillofacial Surgery
College of Dentistry, University of Mosul

الخلاصة

الأهداف: تهدف الدراسة إلى تقييم تأثير مادة بيتا ثلاثي الكالسيوم على التام عظم الفك السفلي للأرانب. **المواد وطرائق العمل:** شملت هذه الدراسة التجريبية عشرة أرانب ، تم قياس أوزانها قبل العملية ثم أعطيت مخدرا عاما حسب الوزن. أجريت العمليات في ظروف معقمة ، ثم عمل حفرتين مستطيلتي الشكل تامة السمك بمقياس 6x5 ملم في كل جانب من الفك الأسفل للأرانب. الحفرة الأمامية تركت بدون معالجة لتكون المجموعة الضابطة والحفرة الخلفية ملئت بمادة البيتا ثلاثي الكالسيوم فوسفيت ، بعد أسبوع من العملية تمت التضحية بخمسة أرانب وبعد شهر من العملية تمت التضحية بخمسة أرانب أخرى ثم تمت معاينة الفك السفلي نسيجيا . **النتائج:** الفحص المجهرى للمقاطع بعد أسبوع واحد للمجموعة التجريبية اظهر تنظيما أكثر ، اقل مستوى من خلايا الالتهابات وكذلك أنسجة منظمة أكثر من المجموعة الضابطة وكذلك اظهر الفحص المجهرى للمقاطع بعد شهر للمجموعة التجريبية اظهر نسيج عظمي أكثر مما لدى المجموعة الضابطة . **الاستنتاجات:** لدى بيتا ثلاثي الكالسيوم فوسفيت القدرة على تسريع التام العظم بعد أسبوع وبعد شهر من وضعه في الفك الأسفل لدى الأرانب.

ABSTRACT

Aims: The present study designed to assess the effects of a bone substitute consisting of β -Tricalcium Phosphate (β -TCP) on acceleration of bone healing of surgically created defects. **Materials and Methods:** Ten rabbits were included in the study. Before the operation, each rabbit was weighed and given anesthesia accordingly. Surgery was performed under aseptic conditions. The left and right mandibles were exposed through a sub-mandibular incision. Two rectangular full thickness defects (10x5 mm) were created in each side. The anterior defect was left untreated to serve as a control while the posterior one was filled with β -TCP in each side. Five rabbits were sacrificed one week after surgery and the other five rabbits were sacrificed one month after surgery. The bony mandibles were dissected from the heads and immediately immersed in 10% formalin solution and sent for histological analyses. **Results:** Microscopic examination of the experimental section of one week group showed more organization, lesser degree of inflammatory cell infiltrate, and more granulation tissue formation than the control group. Similarly, microscopic sections from the experimental osseous defects taken at 1 month interval showed more pronounced callus formation than the control group. **Conclusions:** β -tricalcium phosphate accelerate bony healing at both one week and one month interval which is especially important in dental implants and in oral and maxillofacial reconstruction, for both the functional and psychological aspect.

Key words: β -tricalcium phosphate , bone substitute materials, bone healing.

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INTRODUCTION

Bone defect of varying sizes occurs secondary to surgical treatment of lesions, trauma or deformity and presents a signifi-

cant problem for oral and maxillofacial surgeons and orthopedists.^(1,2) Dehiscence and fenestrations may develop during the preparation for implant placement in nar-

row ridge, which can compromise the mechanical stability of the fixture. In order to solve this problem and other problems due to bone defects autogenous or allogenic bone graft and synthetic bone substitutes have been employed for reconstruction purposes resulting in alveolar ridges with sufficient bone volume.⁽³⁾

Bone graft is often necessary to provide support, fill voids, and accelerate or enhance biologic repair of skeletal defects. Autogenous bone grafting from intra and extra oral sites are considered to be the gold standard for repair of most osseous defects including those in the maxillofacial region that all alternatives must meet or exceed.^(4,5) However, there are limits to the amount of bone that can be harvested from a patient skeleton, autogenous bone grafts may also increase the risk of morbidity such as infection, pain and length of hospital stay associated with the second site surgery.⁽⁶⁾ Allogenic bone is harvested from an individual other than the patient, so concerns exist about the potential for disease transmission like hepatitis and human immune deficiency virus infections as well as limitation of the grafts and occasionally incomplete healing. As a result, allogenic bone is less than ideal as a grafting material⁽⁴⁾. Allograft (which is harvested from one individual and transferred to another of the same species) and xenograft (from another species) usually prevent complete bone ingrowths because they are short of osteogenic cells as well as to the immune reaction to the graft. As a result, there has been recent interest in the development of new grafting materials. An ideal synthetic bone substitute should be tolerated by the host tissue without any adverse reaction, promote bone formation, have appropriate mechanical strength, and be malleable⁽⁷⁾ and completely absorbed and replaced by the newly formed bone.⁽⁸⁾

Beta-tricalcium phosphate (β -TCP) has been used in many experimental and clinical studies. It is a bone substitute that is osteoconductive and biodegradable.⁽⁹⁾ It can assist in the processes of bone regeneration. It resorbs after a certain period of time in situ and is then replaced by newly formed bone, which ensures ideal healing of the defect.⁽⁶⁾ TCP

considered to be biocompatible (not stimulating inflammatory or foreign body giant cell activity). This is mainly because TCP is composed of calcium and phosphate ions, which are the most commonly found elements in bone. However, TCP cements have a slower resorption rate than bone and are usually too dense to allow bone tissue to grow into the defects in a limited period of time.⁽¹⁰⁾

This study assessed the effects of a bone substitute consisting of absorbent β -TCP on acceleration of bone healing of surgically created defects.

MATERIALS AND METHODS

Ten white albino male rabbits weighing between 1.5 and 2 kg were included in this experimental study. All animals were kept outdoor in cages and fed green leaves, fruits, and vegetables. They all seemed to have good health throughout the period of the study. The study protocol was approved by the Scientific Committee of Oral and Maxillofacial Surgery Department.

Before the operation, each rabbit was weighed and given anesthesia accordingly. Each animal was anaesthetized with intramuscular injection of 10% ketamine (40 mg/kg) (ELSaad, Aleppo, Syria) and 2% xylazine (5 mg/kg) (Inferchemie, Holland). Then its fur was removed from the lower border of the mandible. The mandible of the animals was scrubbed thoroughly with butadiene. Surgery was performed under aseptic conditions. The left and right mandibles were exposed through a submandibular incision. Two rectangular full thickness defects (10 × 5 mm) were created in each side using a surgical bur under copious irrigation with normal saline. The anterior defect was left untreated to serve as a negative control while the posterior one was filled with β -TCP in each side. The wound was then closed with 3/0 black silk suture. The rabbits were given intra-muscular injections of oxy tetracycline hydrochloride calculated at 30 mg/kg body weight, immediately after surgery. All animals recovered from anesthesia without complications.

Five rabbits were sacrificed one week after surgery and the other five rabbits

were sacrificed one month after surgery. The bony mandible were dissected from the head and immediately immersed in 10% formalin solution for fixation, for three days, dehydrated in graded alcohol and embedded in paraffin. Five-micrometer decalcified sections were prepared and stained with haematoxylin and eosin.

RESULTS

Histopathological Analysis:

The slides available for histological examination comprised 40 slides, divided into two time groups (one week and one month), control and study defect slides.

Histopathological evaluation of specimens from both control and experimental groups were done with particular emphasis on the degree of inflammatory

process, hematoma formation, organization and callus formation.

Control group at the end of the first week:

Microscopic examination of the sections of the control group after one week of the experiment shows substantial hemorrhage into the osseous defect with scattered inflammatory cells mainly macrophages, accompanied by granulation tissue reaction in the form of proliferation of small sized vascular spaces lined by flat endothelial cells and their lumen filled by blood, associated with pale edematous stroma containing spindle fibroblast cells and macrophages. Evidence of bone necrosis is seen in the form of scattered necrotic bone trabeculae having irregular shape and their lacunae contain dead osteocytes. Focally there is osteoblastic proliferation, as shown in Figure (1).

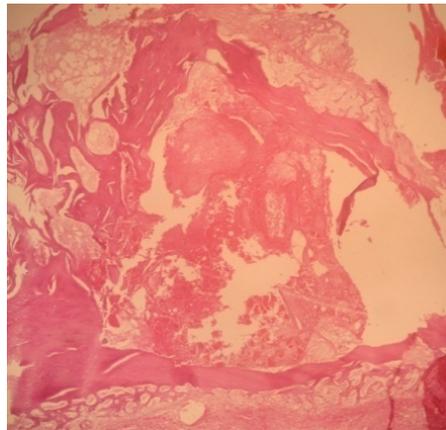


Figure (1): Control specimen after one week. Bone necrosis is prominent. No osteoid formation is seen.

Test group, one week:

Microscopic examination of the sections of the experimental group after one week of the application of tricalcium phosphate in the osseous defect showed more organization in the form of hemorrhagic foci, lesser degree of inflammatory cells infiltrate, granulation tissue formation i.e.

cellular and vascular connective tissue, fragments of necrotic bone trabeculae. However, there is more marked osteoblastic proliferation accompanied by early osteoid formation in the form of trabeculae of woven bone with prominent osteoblastic rimming, Figure (2).

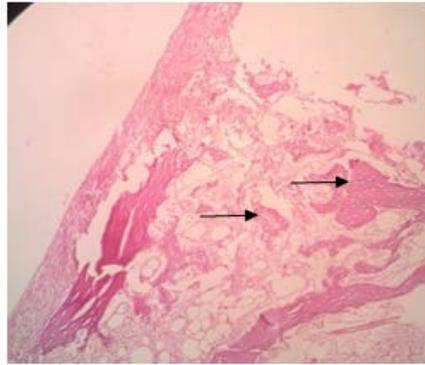
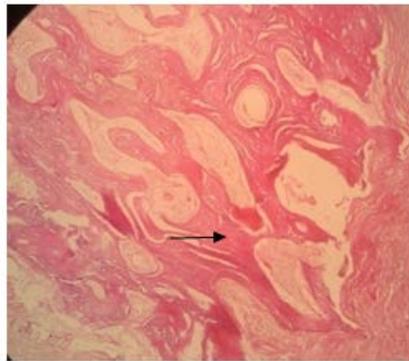


Figure (2): Test specimen after one week. Early osteoid formation is noted with less degree of inflammatory infiltrate.

Control group, one month:

Sections taken at 1 month interval in the control group showed callus formation with irregular trabeculae of woven bone

rimmed by osteoblasts set in a background of fibrotic stroma with little inflammatory infiltrate and no granulation tissue. Figure(3).



Figure(3): Control specimen at 1 month. Callus formation is noted with irregular trabeculae of woven bone rimmed by osteoblasts and separated by fibrotic stroma. Minimal inflammatory infiltrate is seen.

Test group, one month:

Microscopic sections from the osseous defect taken at 1 month interval in the

group treated by tricalcium phosphate showed more pronounced callus formation, Figure (4).

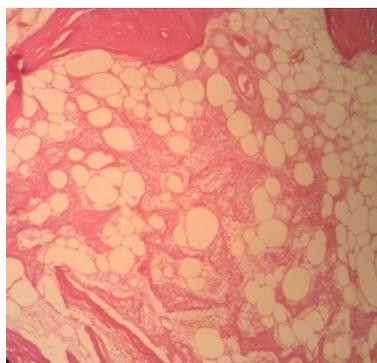


Figure (4): Test specimen after one month. Again callus formation is noted with irregular trabeculae of woven bone. No inflammatory infiltrate or granulation tissue is seen

DISCUSSION

Tricalcium phosphate causes a positive reaction after implantation in terms of bony tissue formation, strengthening or interlocking, which in turn promotes regeneration of the bone and its function⁽⁶⁾. Oi Y et al. in their study at intra bony defects in dogs found that the microstructure of porous β -TCP matrix led to vascularization of the matrix itself (11). Vascularization is important for successful bone regeneration, especially when using a biomaterial because of their role in the nutrition of the migrating cells. A pore size up to 60 micrometer is ideal for vascularization.⁽⁶⁾ The material used in this study had 500-1000 μ m pore size.

The ideal bone substitutes should have biocompatibility, excellent osteoconductive properties and appropriate strength and they should be able to form a suitable shape easily and to ultimately replace the bone completely within a short period. Bone substitutes must be able to make a suitable shape for the bone defect. β -TCP is in a granular form and therefore it can fill in the damaged area in any form.⁽²⁾

β -TCP would have to be osteoconductive in order to be effective as a scaffold, it appears to serve as a nidus for new bone formation. However, β -TCP is not osteoinductive because it does not contain the proteins necessary to induce bone formation (9). It used in this study as the experiment was conducted in an osteogenic environment, as the osteoinduction properties came from the rabbit bone itself.

Mauri et al. concluded that the combined application of osteoinductive enamel matrix derivative and osteoconductive β -TCP did not increase bone formation in rabbit calvarium compared with use of β -TCP alone.⁽⁹⁾ As well some problems have been reported by the use of non or slowly absorbed osteoinductive hydroxyapatite, which is commonly used bone substitute, these problems may include prevention of the establishment of the blood vessels network when the cavity is filled with excessively dense particles, and normal bone marrow is not formed during bone remodeling due to the presence of the non-absorbed particles.⁽⁸⁾ Synthetic β -TCP acts as a scaffold for bone proliferations and is slowly resorbed by

osteoclastic activity and substituted by living bone cells that grow directly in contact with the mineral. One of the most influential factors in the process of β -TCP is macro and microporosity that promotes the ingrowth of blood vessels and enables osteocyte dendrites to infiltrate the micropores⁽¹²⁾. So, β -TCP accelerate osteogenesis merely by functioning as a passive osteoconductive scaffold⁽¹³⁾.

Osteoconduction is a three dimensional process that is observed when porous structures are implanted into or adjacent to bone. Tricalcium phosphate are osteoconductive because osteoblasts adhere to them and deposit bony tissues on their surface. TCP forms a scaffold for closing the bony defect⁽⁶⁾.

Generally bone healing is faster in the TCP group than the control group at 7 days after surgery. This may be attributed to increased calcium and phosphate ions inside the bony cavity following dissolution of the particles.⁽⁸⁾ While Eleftheriadis E et al. in their study found that the osseous defects in rabbits, mandible that were left untreated (control group) had become completely filled with mature bone as soon as the end of the 3rd week, earlier than in the β -TCP group. This observation may have been attributed to the limited dimensions of the prepared osseous cavities which could not be considered as critical size defects⁽¹²⁾. In this study the size of the defects was 10 \times 5 mm. which is a documented critical size defect in rabbits, mandibles⁽¹⁴⁾

In contrast Hirn et al. stated that bone defects that are left empty heal just as when filled with a bone substitute material, it is a merely time related process. Bone substitutes are quite expensive particularly so when used to fill large defects (15). It has been suggested that the repair of bone defects depends on the size of the defect, i.e. a bone defect larger than a certain size (a critical size) cannot be healed completely with bone. The remaining defect is filled with fibrous connective tissues, that is because formation of repaired bone ceased within 6 months in both critical and non-critical size defects regardless of completion of the defect repair.⁽¹⁶⁾

Podaropoulos et al. (2009) created critical size cylindrical bone defects of 10

mm diameter and 5 mm depth. All defects were left to heal for 4 months, he found that in the β -TCP group the defects were partially filled with new bone, the graft particles still dominated the area, and the outer cortex was not restored while in the empty cavity group, incomplete new bone formation was observed, the outer dense cortical layer was restored in a lower level, near the base of the defects.⁽¹⁰⁾

In this study β -TCP accelerate bone healing at a faster time interval one week and 4 weeks which is especially important in dental implants and in oral and maxillofacial reconstruction, for both the functional and psychological aspect.

CONCLUSIONS

When β -tricalcium phosphate is used as a bone substitute filling a bony cavity, it accelerates bony healing at both one week and one month intervals regarding the degree of inflammatory process, hematoma formation, organization and callus formation.

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