



## Effect of $\beta$ -TCP Powder Derived from Biomaterials on Regeneration of the Femoral Bone Defects in Rabbits

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### Abstract

The current study evaluated the bone healing process of the  $\beta$ -TCP powder to treat experimentally induced bone defects in rabbit models. The  $\beta$ -TCP powder was prepared using the chemical method, which is characterized by Field Emission Scanning Electron Microscopy (FESEM), and Fourier Transform Infrared Spectroscopy (FTIR). After that, the  $\beta$ -TCP was converted into powder by applying mechanical compression pressure. Thirty (30) healthy New Zealand male rabbits were divided equally into two groups: the treated group and the control group. The previously prepared  $\beta$ -TCP powder filled the experimentally induced sagittal split fractures at the mid-shaft of the femoral bone in the group treated, while in the control group, the induced bone gaps were left without any treatment. The histopathological results showed normal bone marrow tissue with bone tissue regeneration that extended from both ends of the defect with active osteoblasts toward the powder in the treated group, while in the control group, there was a formation of hyaline cartilage surrounded by trabecular bone along with granulation tissue infiltrated with inflammatory cells, which surrounded the fracture location. The bone marrow examination showed the presence of Megakaryocytes and an increase in erythroid to myeloid in the treated group compared to the control group. The statistical analysis showed a significant increase ( $P < 0.0001$ ) in new bone tissue formation in the treated group as compared with the control group during eight months following surgery. The fibrous tissue formation increased significantly ( $P < 0.0001$ ) in the control group compared to the treated group. Moreover, the current work indicated that the  $\beta$ -TCP powder can be used as a bioactive material for bone tissue regeneration, but it requires more time to be resorbed.

**Keywords:**  $\beta$ -tricalcium phosphate, Seashells, Biomaterials, femoral defect.

### Introduction

The main concern of orthopedic surgeons is to discover new methods that can promote the bone healing process within a relatively short period of time, especially in cases of segmental and critical bone defects caused by bone cysts, bone tumors, or direct trauma [1]. A broad spectrum of biomaterials has been used in the medical field including ceramics, glass and polymers. Bioceramic materials receive a good attention in orthopedic surgery not only for their chemical compositions (calcium and phosphate) that are identical to the inorganic phase of natural bone minerals, but also for their biological activity that can enhance bone tissue formation.

These biomaterials might stimulate osteoblastic cell proliferation and differentiation [2]. Many bioactive ceramics are derived from biological sources that are readily available and cheap to be used in the orthopedic field [3], like  $\beta$ -tricalcium phosphate ( $\beta$ -TCP), which were investigated as bio-material related to their biological activity, biocompatibility and the possibility to adhere to bone tissue after implantation [4]. Recently, natural bone alternative materials, consisting mainly of calcium phosphate materials, are used as a bone powder for bone tissue regeneration. Calcium phosphates are considered one of the main inorganic components of the avian Seashell. Seashell powder is a natural source of calcium and is

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considered a biodegradable material that is widely available as a waste material in nature. In addition to that, Seashell-derived calcium carbonate has been shown to be effective as a biocompatible and osteoconductive bioactive material in many animal types of research. Fractures in the femoral bone are among the most common fractures that occur in clinical cases. There are many risk factors that are associated with femoral fractures, which include osteoporosis or low bone mineral density, nutritional factors, lifestyle and genetic factors [5]. Because the biosorption process of bioceramic powders has not been fully investigated yet in clinical studies, therefore, the current study aimed at evaluating the biodegradable, biocompatible and new bone tissue formation of the  $\beta$ -TCP powder that is used for the treatment of the induced critical size femoral bone defects in rabbits.

## **Material and Methods**

### ***Preparation of Seashell $\beta$ -TCP powders***

The Seashell  $\beta$ -TCP powders were prepared by using a chemical method [6]. At first, the shells were washed with distilled water and then boiled at 100 °C for 30 minutes to remove shell membrane and debris. This was followed by the drying process in that was performed in an oven at 100 °C for 30 minutes also. The Seashell calcium carbonate powder was dissolved in the nitric acid with a concentration of (1 mol/L) with continuous stirring for two hours. Then a drop of  $\text{Na}_2\text{HPO}_4$  was slowly added to the solution of  $\text{Ca}(\text{NO}_3)_2$  on a magnetic stirrer at 50 °C for 2 hours. Then, the precipitate was filtered and washed with distilled water and then left to dry at the room temperature overnight. After that, the product was calcined in a muffled furnace at 1200 °C for two hours to yield  $\beta$ -TCP. Finally, the resultant powder was crushed to a fine powder (10 $\mu\text{m}$ ) with a laboratory mortar grinder (Retech Rm200, India), then the produced  $\beta$ -TCP powder, were stored in sterile plastic container until used.

### ***Evaluation of the powder***

#### **Field Emission Scanning Electron Microscopy (FESEM)**

The FESEM was obtained using 50-FEI (Holland) at an accelerating voltage of 6.68 kV. The FESEM provides the topographical surface of the powder and for the detection and discrimination of emergence of crystals.

#### **Fourier-Transform Infrared Spectroscopy (FTIR)**

The active groups of  $\beta$ -TCP powders were recognized by Fourier transform infrared spectroscopy (FTIR) using (Shimadzu-8400S Japan). The examination was accomplished in the wavelength range from 400  $\text{cm}^{-1}$  to 4000  $\text{cm}^{-1}$ .

#### **Experimental design**

The seashells were obtained from the local market in Tikrit city. The Seashell  $\beta$ -TCP powders were prepared by using the previously mentioned method. All experimental protocols were conducted using the laboratory animal's guidelines and it was proved by the Animal house center at the College of Veterinary Medicine, University of Tikrit. Thirty, three-month old and (2-2.5 kg) weight, healthy New Zealand male rabbits were used in this study. The rabbits were randomly divided into two groups: the control group and the experimental group.

#### **Surgical procedure**

The surgery was carried out using general anesthesia through intramuscular injection of mixing 10% Ketamine HCl (Alfasan, Holland), 2% Xylazine (Bayer, Germany) at a dose of 30 mg/kg and 4 mg/kg body weight separately. The location of surgery was shaved and disinfected with 10% povidone-iodine. After that, the operated fields were incised with 5-6 cm. longitudinal skin incision was made in the left hind limb (Fig.1.A). The blunt dissection was performed through the subcutaneous and muscular layers to expose the femoral bone. Subsequently, a gentle dissection of the thin periosteum, with continuous irrigation with saline solution, a slow electrical osteotomy saw (AXS-II, China) was used to make a sagittal split fractures in the diaphysis of the femoral bone with its attachment periosteum. These fractures were left without any treatment in the control group, while in the experimental group was grafted with previously synthetic  $\beta$ -TCP powder (Fig. 1.B). The muscles and subcutaneous incision were closed using a 2-0 Polyglactin 910 (Unisynth, India) suture material with a continuing pattern. The skin wound incision was sutured with 2-0 silk (Demetech, USA) suture material in a simple interrupted pattern. The hind limb of the rabbits was immobilized for four weeks using plaster casts. Moreover, All the animals received diclofenac sodium at a dose of 3mg/kg and procaine penicillin at a dose of 25 mg/kg intramuscularly for 5 days postoperatively. The animals were sacrificed after eight months for histopathological assessment postoperatively, using an overdose of ketamine HCL.

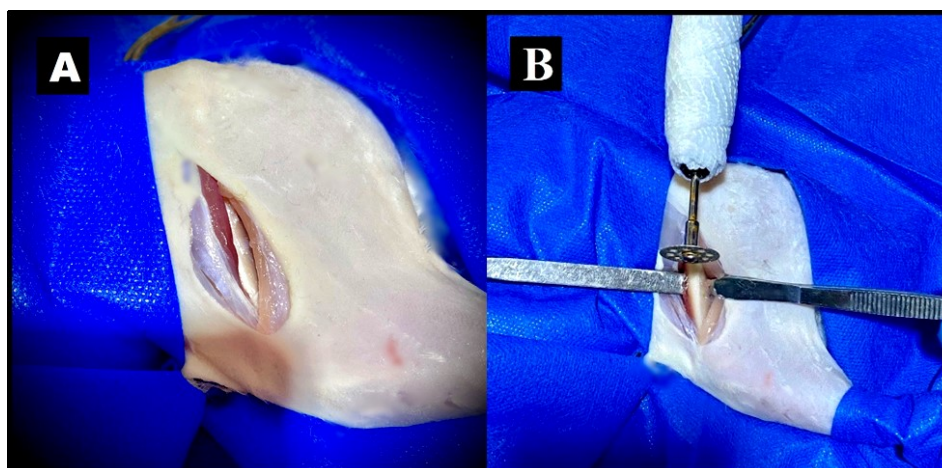


Fig. 1. A. Surgical skin incision was performed in the right hindlimb. B. Stainless steel trephine burr used to create bone defects.

#### Clinical and macroscopically evaluation:

All animals were examined daily after surgery, to check weight-bearing ability, or any signs of wound edema, hyperemia, swelling, or pain on digital palpation. All animals remained in a normal healthy condition throughout the course of the experiment. After 8 months following surgery, the specimens were dissected from the surrounding tissue and grossly examination at the end of the present study was conducted to evaluate the powder bone incorporation (Figure 1).

#### Histopathological evaluations:

The harvested bone specimens with adherent soft tissues were dipped in a solution of 10% solution of buffered formalin. All specimens were decalcified with a solution of 10% nitric acid for two weeks, followed by irrigation with distilled water for 4 hours. After dehydration, all samples were immersed in liquid paraffin. After that, the specimens were sliced into small pieces (5- $\mu$ m-thick) and then stained with hematoxylin and eosin (HandE). Then they were examined by the light microscope (Leica, China), to evaluate the bone healing processes grades at the defect spot by using the semi-quantitatively analyze scores according to modify (7) shown in (Table1).

TABLE 1. Histopathological grade scores.

Index name	Score level	Description
Newly bone	0	No grade of newly formed bone
	1	$\leq 10\%$ of the original bone defect
	2	$\leq 20\%$ of the original bone defect
	3	$\leq 30\%$ of the original bone defect
	4	$\leq 40\%$ of the original bone defect
Newly connective Tissue	5	$\leq 50\%$ of the original bone defect
	6	Full healing
	7	$\leq 10\%$ of the original bone defect
	8	$\leq 20\%$ of the original bone defect
	9	$\leq 30\%$ of the original bone defect
Newly cartilage	10	$\leq 40\%$ of the original bone defect
	11	$\leq 50\%$ of the original bone defect
	12	$\leq 60\%$ of the original bone defect
	13	$\leq 70\%$ of the original bone defect
	14	$\leq 80\%$ of the original bone defect
Newly cartilage	15	$\leq 90\%$ of the original bone defect
	16	$\leq 100\%$ of the original bone defect
	17	No grade of hypertrophic cartilage.
	18	$\leq 10\%$ of the remnant defect
	19	$\leq 20\%$ of the remnant defect
Newly cartilage	20	$\leq 30\%$ of the remnant defect
	21	$\leq 40\%$ of the remnant defect
	22	$\leq 50\%$ of the remnant defect

#### Immunohistochemistry

Immunohistochemistry was also performed to identify primary osteocytes. staining on rabbit femur bone. Paraffin slices were taken from the middle of the femur bones of the 8-month-old rabbits and then immunohistochemistry was conducted. After being submerged in 10% formalin, decalcified with 14% EDTA, paraffin-embedded and sectioned on a microtome, the tissue was finally frozen for future study. The sections were stained with a VECTASTAIN Elite Kit, Peroxidase (Standard) (Vector Lab, USA), product code PK6100, which included applying the primary and secondary antibodies directly to the tissue at 1:250 dilution.

After methyl green counterstaining the slides were mounted for digital microscopy photography (Leica, DFC, USA).

#### Bone marrow examinations

Bone marrow was aspirated from the margins of bone-powder contact in both groups and stained with Giemsa stain.

#### Statistical analysis

The information was presented in the form of arithmetic means and standard deviations (SD). When a sample t-test has a p-value of less than 0.05, the results are considered significant. GraphPad Prism version 8.0.1 was used to conduct all statistical analyses.

## Results

### FESEM

The FESEM of the powder particles at magnification (10 and 20 $\mu$ m) appear as hexagonal plates and needle-like crystals agglomerated in irregular morphology with microporous that ranges between 5 and 10 $\mu$ m (Fig. 2).

### FTIR

The active groups of the  $\beta$ -TCP powder were confirmed by FTIR spectra (Fig. 6), which shows the bands at 560, 680, 1030, and 1090  $\text{cm}^{-1}$ , are attributed to the stretching and deflections modes of the phosphate ions  $\text{PO}_4^{3-}$  groups, and the bands at 730 and 1220  $\text{cm}^{-1}$  were represented to  $\text{P}_2\text{O}_7^{4-}$  groups (Fig. 3).

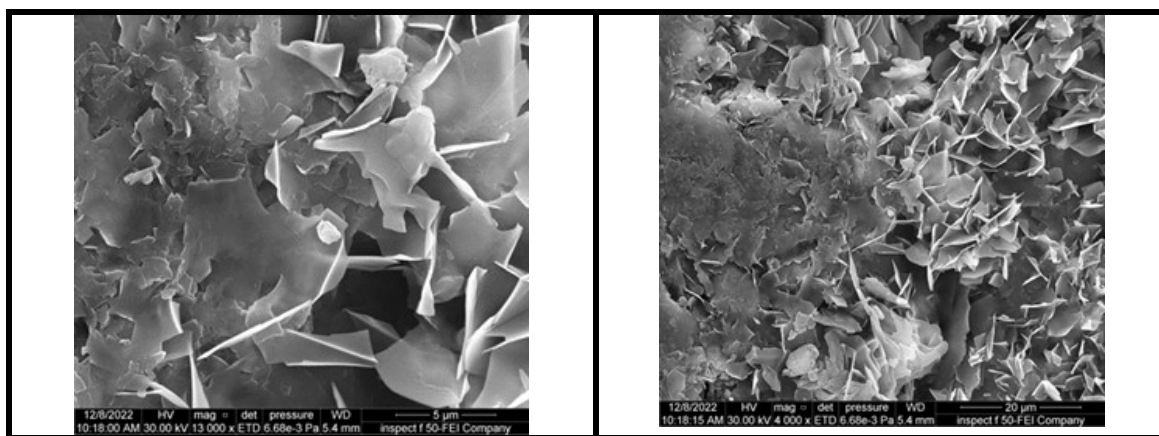


Fig. 2. FESEM of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) powder at magnification 10 and 20 $\mu$ m.

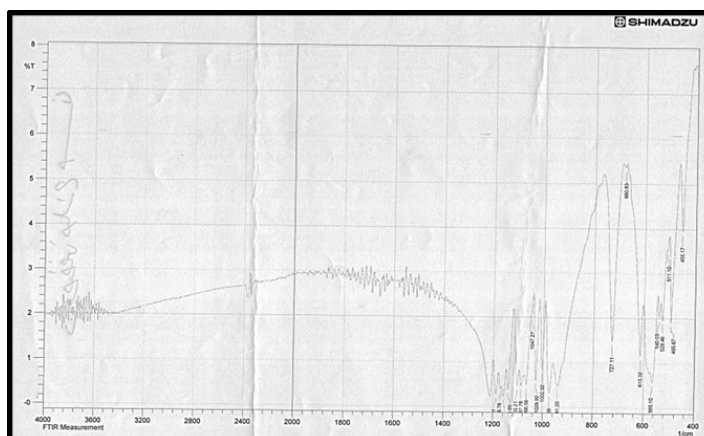
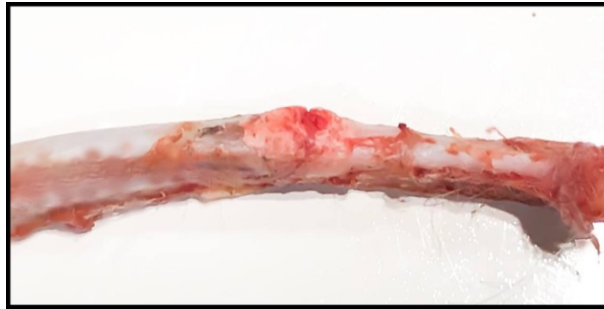


Fig. 3. Spectra of FTIR starting powder and samples obtained after a reaction time of 2 hours at 1200C°.

**Clinical and macroscopical evaluations:**

The clinical observation was carried out for all animals with regular observation up to the first months post-surgery and no signs of wound infection, inflammation, edema, or abscess formation were evident. All animals could gain weight during the first three weeks after surgery. Moreover, all the

animals have a normal appetite during the experimental period. The macroscopical finding at eight months post-surgery revealed that the powder appeared as a white-colored dense material that firmly incorporated to both ends of the femoral defect and this indicates a good bonding between the powder and the bone (Fig. 4).



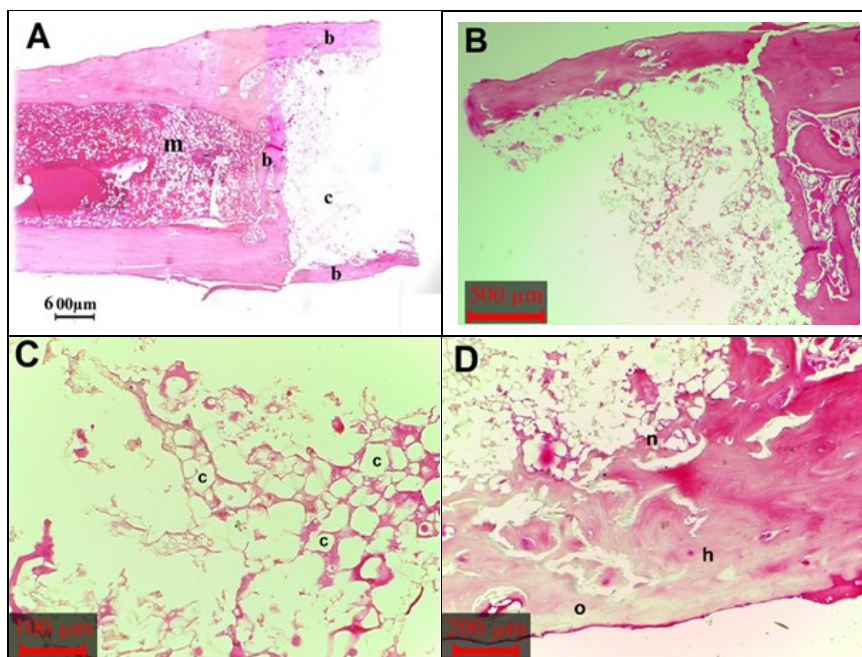
**Fig. 4. The cross section of the femoral bone defect grafted with  $\beta$ -TCP bone powder after 8 months of the sacrifice of the animal.**

**Histopathological evaluations:**

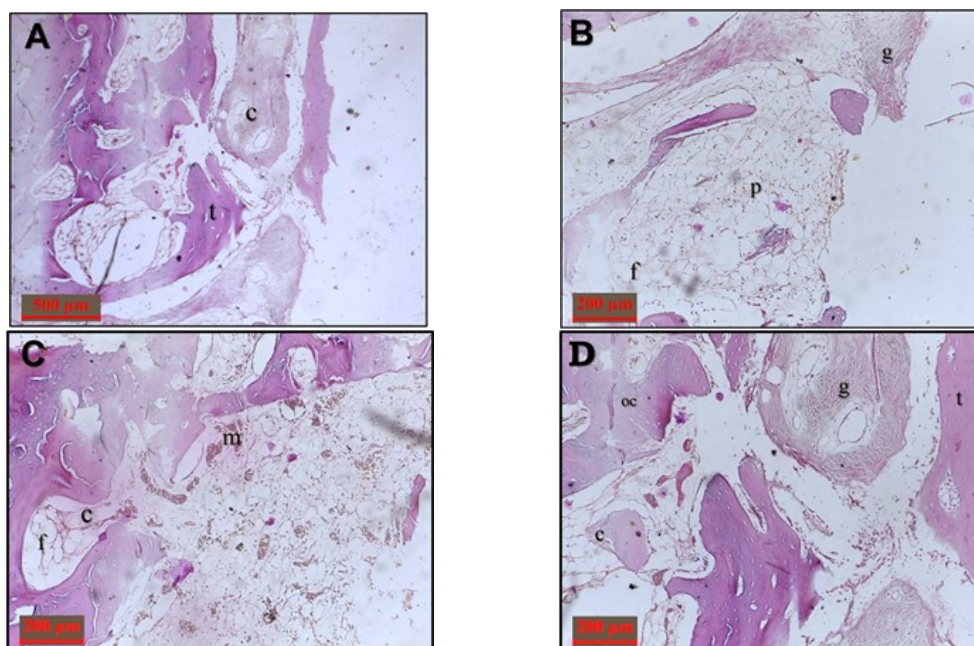
Histopathological sections in the experimental group showed that normal bone marrow tissue was present with the bone tissue regeneration extended from both ends of the defect with active osteoblasts toward the powder (Fig. 5, A) and very thin periosteum and endosteum were observed along with thick compact bone with a closed medullary cavity at bone-powder contact between the bone and the powder (Fig. 5, B). The network of tissue trabeculae extends from the edges of the new bone of the defect into the porous material of the powder (Fig. 5, C). The new bone formation invaded the materials from the edge to the center of the powder, with numerous osteocytes that are randomly embedded within the lacunae observed at the edge of the powder with large

Haversian canals with newly formed bone were presented in the peripheral region of the powder (Fig. 5, D).

In the control group, the histopathological sections eight months post-operation show a hyaline cartilage surrounded by trabecular bone that demonstrated granulation tissue infiltrated with inflammatory cells surrounded by the site of fracture (Fig. 6, A, and B). The formation of spongy bone was infiltrated with fat cells and surrounded by trabeculae along with neovascularization and inflammatory cell infiltration also still the presence of the trabecular bone that contains active osteoblast with large spaces filled with granulation tissue and (Fig. 6, C and D).



**Fig. 5. Histological sections of critical- sized bone defect in experimental group repair with CPP powder at 8 months after implantation stained with hematoxylin and eosin. Shows (A) bone matrix surrounded by edges of new bone formation with closed the medullary cavity. (B) The edge of new bone formation extended from both ends of the defect toward the powder with a complete closing of the marrow cavity. (C) Powder mineral crystals surrounded by new bone formation. (D) presence of osteocytes within the lacunae along with the presence of the Haversian canal. Abbreviation: (b) new bone formation. (m) medullary cavity. (c) powder. (o)osteocytes. (h) Haversian canal.**



**Fig. 6. Histological sections of the critical- sized bone defect without treatment in the control group at 8 months after implantation stained with hematoxylin and eosin. Show: (A) Abbreviations: (t) trabecular bone. (p) Polymorphonuclear cells. (c) Hyaline cartilage. (f) fat cells. (g) granulation tissue. (oc) osteocytes.**

#### Immunohistochemistry

The result of immunohistochemistry showed the cell movability towards the matrix of the powder. Which showed high expression of osteoblast and embedding primary osteocytes (Fig. 7).

#### Bone marrow examinations

After the complete stain of the bone marrow smear, otherwise the presence of megakaryocytes (Fig. 8, A) and increase myeloid the o erythroid ratio (Fig. 8, B) as compared with the h control group (Fig. 8, C).

The histopathological analysis showed a significant difference between the two groups ( $P < 0.0001$ ) in the newly formed bone tissue in the experimental group at a level of  $\leq 50\%$  of the original bone defect compared to the control group that has a value of about  $\leq 20\%$  of the original bone defect eight months after the operation. The fibrous tissue formation significantly increased ( $P < 0.0001$ ) become

$\leq 80\%$  of the original bone defect in the control group compared to the experimental group which has a value of  $\leq 50\%$  of the original bone defect. The newly formed cartilage showed a significant increase ( $P < 0.0001$ ) in the control group which represented about  $\leq 50\%$  of the remnant defect compared to the experimental group, which was a value of  $\leq 10\%$  of the remnant defect (Fig. 9).

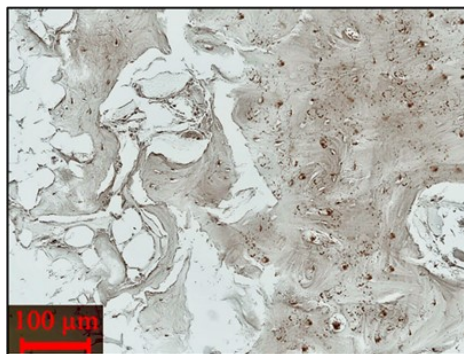


Fig. 7. Immunohistochemical staining can be identified by the dark brown staining of the osteocyte cell bodies and cell processes within the canaliculi.

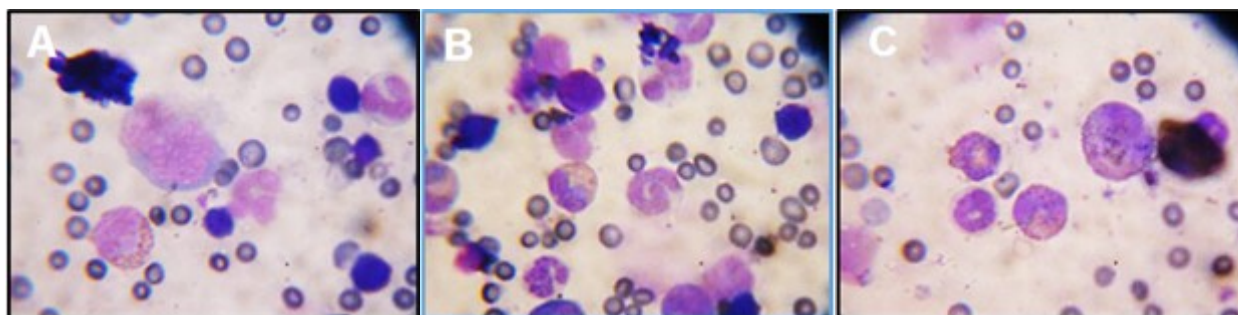


Fig. 8. (A) Bone marrow smear shows the presence of megakaryocytes. (B) Bone marrow smear shows an increased myeloid to erythroid ratio. (C) Bone marrow smear shows normal myeloid to erythroid ratio, (Giemsa stain X).

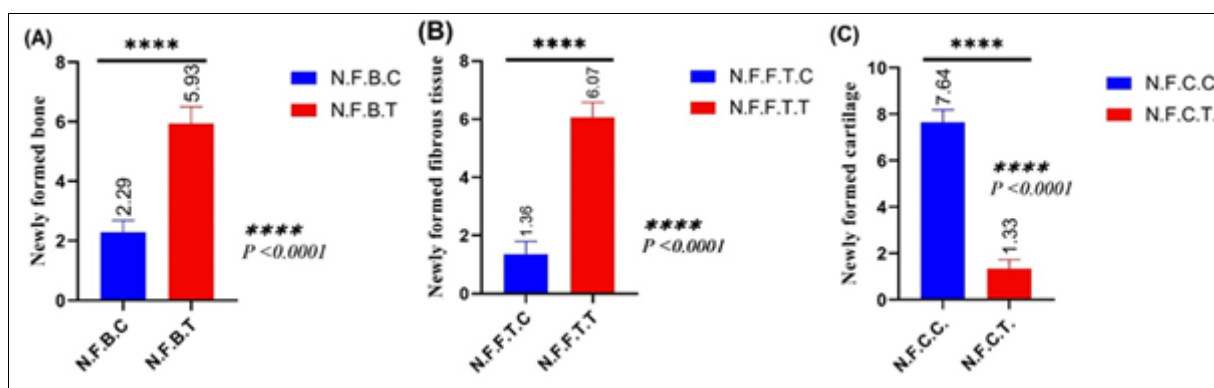


Fig. 9. Significant differences were observed between the two groups in terms of the newly formed bone. The values were represented as mean  $\pm$  S.E (n=15) in the statistical analysis that was conducted using the t-test at likelihood level of  $P < 0.0001$ . Newly formed bone control group (N.F.B.C), newly bone tissue experimental group (N.F.B.T), newly formed fibrous tissue control group (N.F.F.T.C), newly formed fibrous tissue experimental group (N.F.F.T.T), newly formed cartilage control group (N.F.C.C.), and newly formed cartilage experimental group (N.F.C.T.).

## Discussion

In bone defects it is usually required to repair the tissue damaged due to a disease, trauma, or orthopedic surgery. Recently, the natural  $\beta$ -tricalcium phosphate powder was extremely researched in orthopedic bone and dental surgery as tissue engineering biomaterials [8], due to its biocompatibility and its similarity to the inorganic phase of the bone tissues [9]. Many studies agree that bioceramics can activate the osteoblastic cell proliferation and biomaterials are always considered as cell carrier powders in bone tissue engineering [10]. Recent studies suggest that  $\beta$ -tricalcium phosphate can stimulate the bone tissues *in vivo*. It has osteostimulative behavior that enables increase the osteogenic activity of mesenchymal stem cells (MSCs) and thus can increase bone tissue formation [11]. The  $\beta$ -TCP powder is considered biocompatible materials, but it demonstrates slow bone formation *in vivo*. The FTIR of the current study shows a highly sharp as well as intense bands presented between  $729$  and  $1215\text{cm}^{-1}$  due to  $\text{P}_2\text{O}_7^{4-}$  groups, that indicates the presence of calcium pyrophosphate. Likewise, the FTIR resulting spectra were in conformity with the previous characteristic  $\beta$ -TCP mentioned by . It is reasonable that the sharp intensity peaks of the absorption bands is considered a good indication to the degree of crystallinity [12]. Many studies established the relationship between the crystal size and porosity on cell growth and bone tissue formation [13]. The FESEM results showed that the porous size ranges between  $5\text{-}10\ \mu\text{m}$ . Similarly, other studies indicated that the size of porosity on the CaP surface ( $<50\ \mu\text{m}$ ) is considered as micropores [14, 15]. Moreover, a previous study proposed that the osteoconductive properties of powder ceramics can be improved by increasing the microporosity [16, 17]. Furthermore, researchers showed that the micropores can enhance the local mechanical properties of powders. Several studies suggested that microporous powders require additional space for bone formation and the cells that penetrate the ceramic matrix prefer pore sizes larger than  $100\ \mu\text{m}$  [18]. Other studies suggested that bone formation with microporous powder involvement in ceramic resorption and then followed by deposition of osteoid [19, 20].

Additionally, the favorable bone growth was in a macropore powder with porous size greater than  $100$  microns [21]. For instance, it is suggested that the microporosity powder of less than  $10\ \mu\text{m}$  porous size requires longer time for resorption degradable and new bone formation because its surface was difficult to be penetrated by the bone-forming cells in terms of cell orientation, adhesion and proliferation. According to the result of the FTIR spectra, the functional groups available in the  $\beta$ -TCP powder can increase powder incorporation with bone

osteogenesis, through  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions are released from the surface of the powder, that causes physical response of cells and tissues to the surface of powder [22]. Therefore, it is established that calcium phosphate-based bioceramics powders consider the biocompatible materials in another orthopedic field, which can enhance new bone formation on their surface, especially in the case of repairing critical-sized bone defects.

Generally, the bioactive powders can assist bone marrow stem cells and osteoblast cells in attachment, proliferation and differentiation [23]. The fusion between the powder and bone tissue depends mainly on various properties that include the primary adhesion between cells and bone powders, followed by cellular responses to the materials under the action of bioactive ions from powder-induced osteogenic differentiation [7]. The current results showed the presence of megakaryocytes that indicate new bone formation. These results were in agreement with the results of [24], who found that megakaryocytes can stimulate the production of new bone by fusion of angiogenesis and osteogenesis together and secrete TGF-1. Also, the results presented show that the increase in myeloid to erythroid. Therefore the same results were documented by [25], who reported that the myeloid cells increase is essential to speed up repair of the bone fracture.

On the other hand, in the current study, the histopathological sections indicated that the bone tissue was growing around the edges of the bone defect and the powder surface, but this integration will not be incomplete even after the 8 month-period after the operation. The new bone formation occurred not only at the edges of the bone but also in the central and peripheral sites of the  $\beta$ -TCP powder. Moreover, the new bone tissue formation direction was from the cut ends of bone toward the powder and this is in conformity with the results of the present study, in which bone tissues extended towards the powder materials. In our results, the statistical outcomes reveal the quantity of bone tissue in  $\beta$ -TCP powder growth with the extent of the implantation time [26]. The results also demonstrated that the  $\beta$ -TCP powder improved the critical defect size but with slow osseointegration properties of the powder, which is considered very significant for the complete reconstruction of bone defects [27]. However, the histopathological result show that the  $\beta$ -TCP powder permits the bone tissue to grow at the margin of the bone defects, but the rate of ingrowth with time was quite little in eight months and the powder was almost not completely degraded [28]. In addition to that, the histopathological sections showed that the osteoblast and osteocytes cells were observed in contact with the edges of bone ingrowth and this indicates that the powder was bioactive and has osteoinduction properties. These results also are in conformity with the previous study [29].



Osteocytes regulate the adaptation of bone to changes and regulate the load on the skeleton. Upon detecting the mechanical load through their canalicular processes, they control a variety of biochemical signals that control the activities of osteoblasts and osteoclasts in order to increase the bone tissue mass. Primary osteocytes express desmin with a polarity that points them in the direction of the matrix, which may assist the integration of osteocytes with the bone matrix [30]. It was found that the primary osteocytes in the immunohistochemistry section suggest the presence of functional bone cells with the active bone matrix that is embedded within the powder. Due to their intraosseous location, osteocytes have a restricted supply of oxygen and nutrients [28]. Consequently, well-embedded osteocytes in the matrix show the secretion of sclerostin, which is considered a potent inhibitor of bone formation [31].

Other studies stipulated that the rate of bony ingrowth depends mainly on the fabrication of the pore and crystals size [32], but the mechanism of implant resorption is not completely understood yet, because it depends on many factors such as host cellular, chemical and physiologic mechanisms. The results of the present study were in conformity with the previous study that illustrated incomplete resorption of the  $\beta$ -tricalcium phosphate powder [33]. In the study, the researchers illustrated the insufficiency of the bone powder in the improvement of new bone formation because of the deposition of obvious new bone formation around the powder materials. Furthermore,  $\beta$ -tricalcium phosphate powders can stimulate bone tissue formations and therefore they have osteoconductive and osteoinductive properties. Additionally, histopathological outcomes showed that  $\beta$ -tricalcium phosphate crystals can attract the bone cells and lead to initiate a strong adhesion between the powder surface and the bone defect ends. This adhesion is associated with the development of a bone-like apatite layer on the powder which allows the new bone to grow and changes its direction to the powder surface through this layer [34]. Also, it has been established that the release of the ions, especially  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  from the powder surface, which can activate the osteogenic process, results in increased osteoblast proliferation [35].

### Conclusion

The  $\beta$ -tricalcium phosphate powder was conducted in rabbits midshaft femoral bone defects to estimate its *in vivo* degradation and bone regeneration. The current study showed that the powder was biocompatible and capable of inducing new bone formation along the edges of the bone defects with the absence of cartilage formation in comparison to the control group. From the other

hand, the resorption rate after a period of eight months was very low in both experimental and control groups. The current study indicated that the  $\beta$ -tricalcium phosphate powder was non-biodegradable over a period of eight months, depending on synthetic conditions, porosity, implantation location and animal species. It is preferred to continuously design the surface microstructure of the powder for the appropriate porous size that is favorable for bone cell alignments and adhesion to enable the formation of bone tissues.

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The authors declare that the present study has no financial issues to disclose.

### Conflict of interest:None

### Authors contributions

Ali Ghazi Atiyah: Practical work, Maher Saber Owain: statistical analysis and Mustafa Salah Hasan: Manuscript writing and editing.

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### تأثير مسحوق $\beta$ -TCP المشتق من المواد الحيوية على تجديد عيوب عظم الفخذ في الأرانب

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قامت الدراسة الحالية بتقييم عملية شفاء العظام لمسحوق  $\beta$ -TCP لعلاج عيوب الحجم الدرجة المستحثة في الأرانب. تم تحضير مسحوق  $\beta$ -TCP باستخدام الطريقة الكيميائية التي تتميز بـ FESEM و FTIR. بعد ذلك، تم تحويل مسحوق  $\beta$ -TCP إلى مسحوق عن طريق تطبيق ضغط الضغط الميكانيكي. تم تقسيم ثلاثين (30) من ذكور الأرانب النيوزلندية السليمة بالتساوي إلى مجموعتين: المجموعة التجريبية والمجموعة الضابطة. ملأ مسحوق  $\beta$ -TCP الفخد مسبقاً كسور الانقسام السهمي المستحثة تجريبياً في منتصف عمود عظم الفخذ في المجموعة المعالجة، بينما في المجموعة الضابطة، تُركت فجوات العظام المستحثة دون أي علاج. أظهرت النتائج التشريحية المرضية أنسجة نخاع عظمي طبيعية مع تجديد أنسجة عظمية تمتد من طرفي الخلل مع خلايا عظمية نشطة نحو المسحوق في المجموعة المعالجة، بينما في المجموعة الضابطة، كان هناك تكوين غضروف زجاجي محاط بالعظم التريبيقي مع الأنسجة الحبيبية المتسربة بالخلايا الالتهابية، والتي تحيط بموقع الكسر. أظهر فحص النخاع العظمي وجود الخلايا النوية وزيادة في الكريات الحمر إلى النخاع الشوكي في المجموعة التجريبية مقارنة بالمجموعة الضابطة. أظهر التحليل الإحصائي زيادة معنوية ( $P < 0.0001$ ) في تكوين الأنسجة العظمية الجديدة في المجموعة التجريبية مقارنة بالمجموعة الضابطة بعد 8 أشهر من العملية. زاد تكوين الأنسجة الليلية معنوية ( $P < 0.0001$ ) في المجموعة الضابطة مقارنة بالمجموعة التجريبية. علاوة على ذلك، أشار العمل الحالي إلى أنه يمكن استخدام مسحوق  $\beta$ -TCP كمادة نشطة بيولوجياً لتجديد أنسجة العظام، ولكنه يتطلب مزيداً من الوقت حتى يتم إعادة امتصاصه.

**الكلمات الدالة:** بيتا ثلاثي فوسفات الكالسيوم، الأصداف البحرية، المواد الحيوية، عيب الفخذ.