

## Densitometric and Histomorphometric Analysis for Testing New Bone Substitutes (BLUE BONE®), a Comparative Study on Rabbits' Femoral Defect

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### الخلاصة

**الاهداف:** تهدف الدراسة الى اختبار قابلية تكوين العظام بواسطة مادة جديدة ذات دقائق صغيرة جدا المادة مكونة من مزيج من مادتين هما الهيدروكسي ايتايت والفسفات ثلاثي الكالسيوم بنسب ٨٠ % للهيدروكسي ايتايت و ٢٠% للكالسيوم ثلاثي الفوسفات. **المواد وطرائق العمل:** في هذه الدراسة تم اختبار عشرون ارنبا محليا بطريقة عشوائية تم تخدير كل ارنب وادخاله عملية جراحية لتكوين حفرتين في عظم الفخذ واحدة منهما وضعت فيها المادة والاخرى تركت فارغة تم تقسيم الارانب الى اربع مجاميع على فترات زمنية مختلفة لقياس حجم الالتئام خلال ثلاث ايام، سبعة ايام، اربعة عشر يوما وثمان وعشرون يوما. **النتائج:** كانت هنالك فروقات معنوية احصائية بين المجموعتين خلال الفترات الزمنية الاربعة. **الاستنتاجات:** نستنتج من ذلك ان المادة التي تم استخدامها كبديل للعظم فعالة في تكوين العظم بكمية أكبر وخلال فترة زمنية أقصر.

### ABSTRACT

**Aims:** to evaluate the osteogenic regeneration ability of new nanometric biphasic hydroxyapatite and tricalcium phosphate (80/20) BLUE BONE. **Materials and methods:** twenty domestic rabbits were used in the present study with two defects were created in the rabbit's femur, one filled with BLUE BONE material and the other defect were left empty as negative control. Densitometric and histomorphometric analysis measured at 3 days, 7 days, 14 days, and 28 days. **Results:** showed positive osteogenic properties of the BLUE BONE due to the osteoconduction properties. **Conclusions:** this study supports that BCP may have better application prospects for bone repair.

**Keywords:** Bone Substitutes, osteogenic regeneration

Zaker KA., Delemi ZH., Taqa AA. Densitometric and Histomorphometric Analysis for Testing New Bone Substitutes (BLUE BONE®), a Comparative Study on Rabbits' Femoral Defect. Al-Rafidain Dent J. 2020;20(2):221-232.

DOI: [10.33899/rden.2020.127184.1035](https://doi.org/10.33899/rden.2020.127184.1035) ©2020, College of Dentistry, University of Mosul

**Received:** 22/5/ 2020      **Sent to Referees:** 30/5/ 2020      **Accepted for Publication:** 25/ 6/ 2020  
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### INTRODUCTION

Regeneration of bones is a daily faced problem in dentistry. Musculoskeletal diseases

rise from trauma, surgical interventions and diseases regarded as second largest disabilities throughout the world as recognized by World

Health Organization (WHO) <sup>(1)</sup>. About 1.2 million people lose their life due to the lack of bone reconstruction facilities <sup>(2)</sup>. Different biomaterials have been introduced for bone regeneration purpose. Among all these biomaterials autogenous bone graft is the gold stander because it has the ideal requirements for bone regeneration as osteoinduction, osteoconduction and the availability of osteoprogenitors cells <sup>(3,4)</sup>. All biomaterials share the same property of osteoconduction <sup>(5)</sup>. There are many types of alloplastic biomaterials that has been introduced among these biomaterials the biphasic calcium phosphate (BCP) show promising results <sup>(6,7)</sup>. Hydroxyapatite and tricalcium phosphate are the most calcium ceramic applied as bone graft <sup>(8,9)</sup>. BCP composed of hydroxyapatite and tricalcium phosphate of different ratios. The sapience of this combination is to produce material with optimum dissolution rate obtained from highly resorption rate of TCP to

replace by new bone <sup>(10,11)</sup> and slowly degradable HA for providing mechanical support under load and to maintain volume <sup>(1)</sup>. BLUE BONE is alloplastic biomaterial composed pf HA/TCP of ratio of 80/20 characterized by nano-metric particles ranged from 195.5 nm-348.2nm.

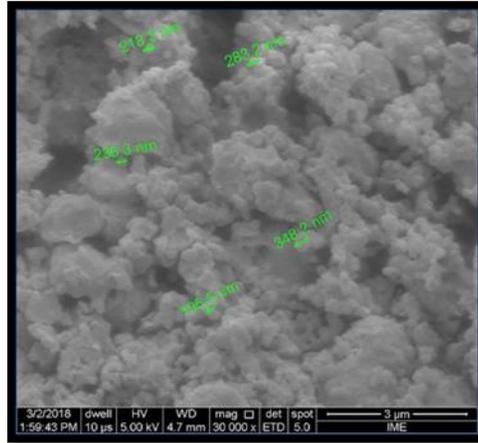
The aim of this study is to evaluate the bone regeneration capacity of the new biomaterial BLUE BONE.

## MATERIALS AND METHODS

The study was accomplished at Mosul University College of dentistry. Twenty rabbits weighted 1.3-1.5 Kg and aged 3-4 months were chosen. The blue bone® graft is a synthetic compound of 80% hydroxyapatite and 20% tricalcium phosphate. Blue bone consists of nanometric particles with homogeneous shapes and sizes (thickness and height) as shown in (Figures1 and 2).



Figure (1): Blue bone material package



**Figure (2):** Scanning electron microscope of blue bone material.

Housing and feeding for all rabbits were the same and all rabbits were examined by veterinary physician to check the animals health condition. Each rabbit was given general anesthesia. It was a mixture of ketamine of 0.6mg/kg and Xylazine of 0.3ml/kg injected intramuscularly. Few minutes later, the animal had lost its consciousness. Then the rabbit positioned on his left side and the area over the right femur shaved and cleaned with povidone iodine. A small of 1.5 cm created over the femur bone near its head by surgical blade no.15 avoiding any trauma to muscle after that the femur bone exposed by blunt dissection. Two holes of 2 mm dimensions depth and diameter created under copious irrigation with distilled water in the femur using 2 mm carbide bure connected to slow motion dental engine. About 5 mg of BLUEBONE material were mixed with drop of distilled water to create pasty material for better application, one hole

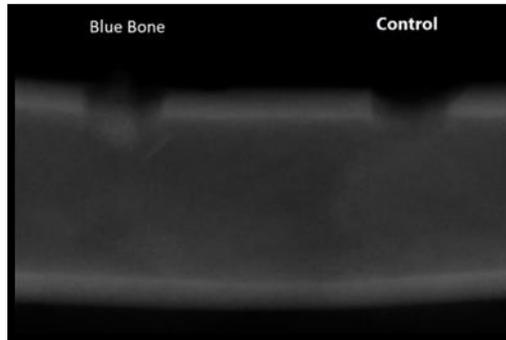
was filled with material and the other left empty. The rabbits were left to heal at different time intervals. Animals were divided into 4 groups and sacrificed at different time intervals at 3 days, 7 days, 14 days, and 28 days. After the end of each time interval, the rabbits at each group had been sacrificed and the femoral bone was isolated and cut into two pieces, one contained the control defect and the other contained the treated defect each defect radiographed at standard alignment and distance from the X-ray source, the radiographic digital system was Carestream®. The setting of the machine was 60 kV, 10 mA and 0.30 seconds. Measures were managed by drawing line from the cortical bone crossing the defect by Cs imaging software 7.0.3. Each specimen kept in formalin 10%, labeled and sent for histological preparation and examination by specialist.

Statistical analysis done by using SPSS program version 26, Data were presented as means  $\pm$  SE (standard error) of mean and analyzed by independent T-test at significant level  $< 0.05$ .

## RESULTS

### 1. Radiographic results

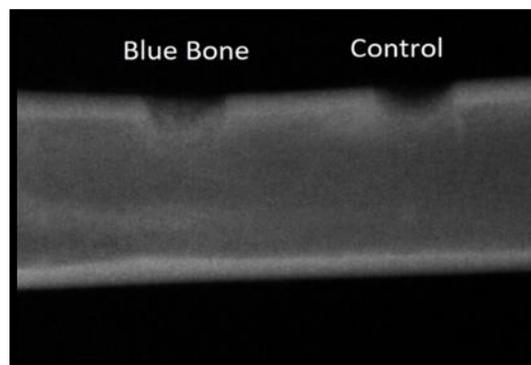
The radiographic results showed an increased radioopacity of treated group as compared with the periods of study. At 3 days after surgery, both defects recognized a higher radioopacity of treated group. As shown in (Figure 3).



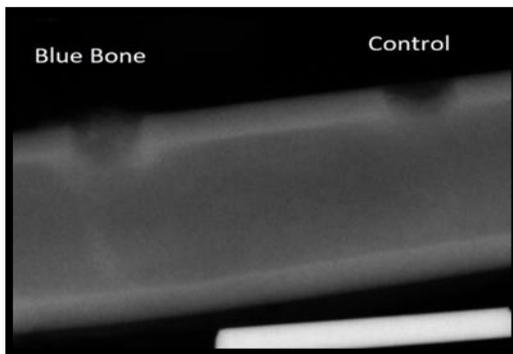
**Figure (3):** Treated and control defects at 3 days after surgery.

At 7 days after surgery, the control defect borders were clearly detected, whereas the treated defect showed obstructed borders as shown in (Figure 4). At 2 weeks after surgery, the control group borders were still detected, while the borders of treated group barely

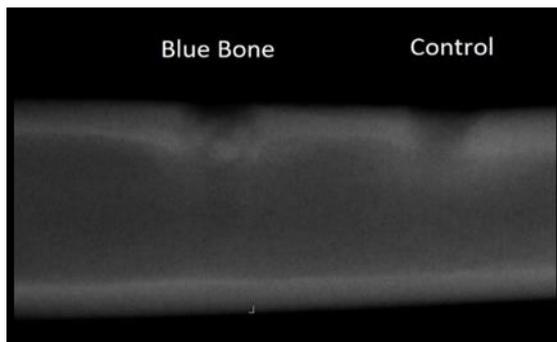
detected with Material still detected as shown in (Figure 5). At 28 weeks after surgery, the borders of both groups were not detected, and the treated group showed higher radioopacity as shown in (Figure 6).



**Figure (4):** Treated and control defect at 7 days after surgery.



**Figure (5):** Treated and control defects at 14 days after surgery.

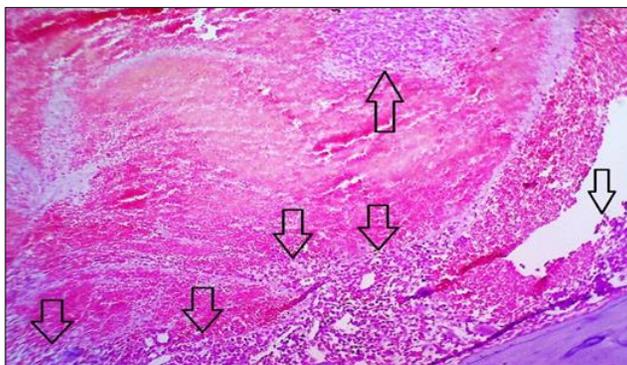


**Figure (6):** Treated group at 28 days after surgery.

## **2.Histological results**

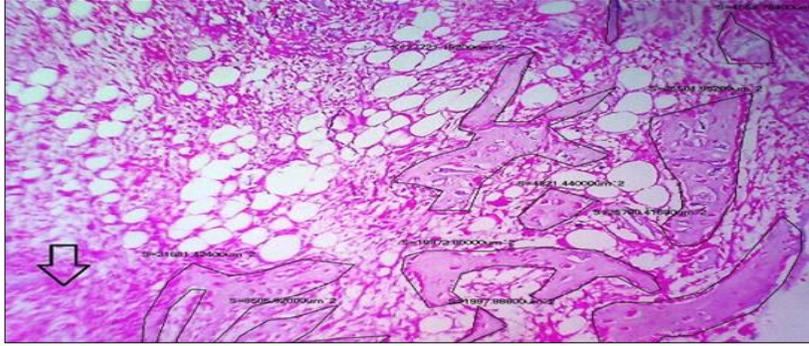
At 3 days after surgery control defect showed sever infiltration of large number of inflammatory cells, no bone spicules found. Granulation tissue were formed with new vascularization. While the defect treated with

BLUE BONE showed defect filled with granulation tissue and moderate infiltration of inflammatory cells. Small bone spicules were formed with good vascularization and the biomaterial still founded as shown in (Figures 7 and 8).



**Figure (7):** Histological section of control group 3 days after surgery at 10 X magnification. Arrows show inflammatory areas.

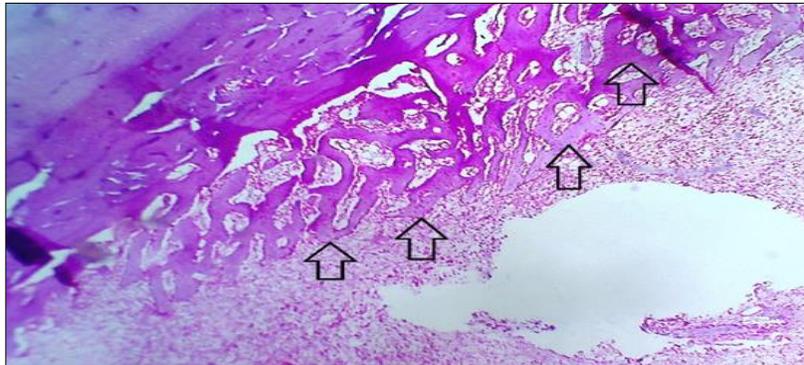




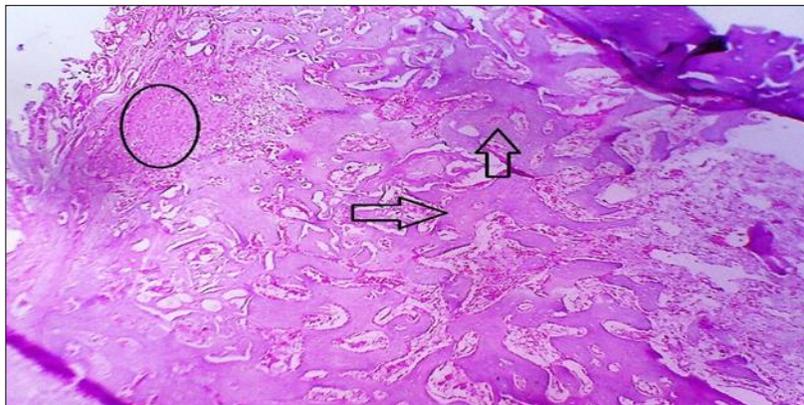
**Figure (10):** Histological section of 10X magnification of BLUE BONE material group at 7 days. The selected areas show the newly formed bone trabeculae. The arrow shows the biomaterial.

At 14 days after surgery, well recognized bone trabeculae were found in the defect area with granulation tissue, no inflammatory infiltration was seen. The treated defect showed a very

well-formed bone trabeculae the biomaterial still found with no inflammatory cells infiltration as shown in (Figures 11 and 12).



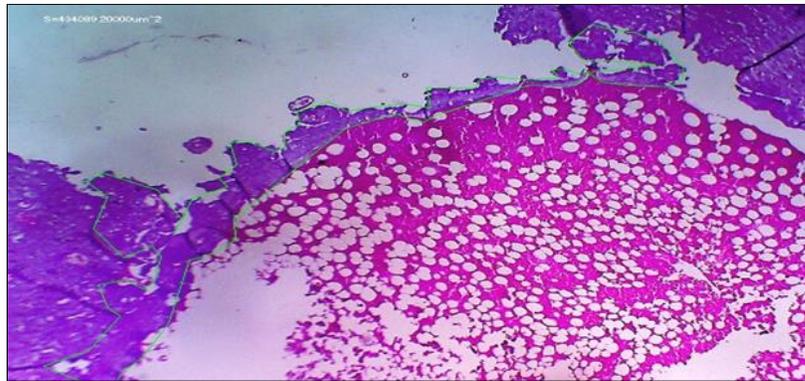
**Figure (11):** Histological section of 4x magnification of control group at 14 days. Arrows show the newly formed bone.



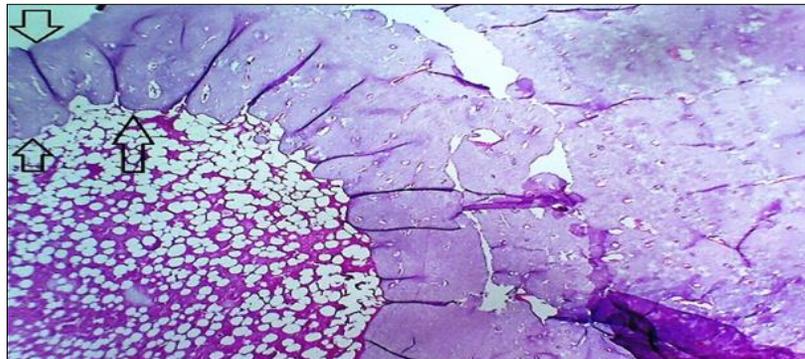
**Figure (12):** Histological section of 4X of BLUE BONE material group at 14 days. The arrows show the newly formed bone trabeculae. The circle shows the remaining biomaterial.

At 28 days after surgery, formation of new compact, bone that closed the defect with no inflammation seen. The treated defect showed

formation of thick compact bone that close the defect area with no inflammation founded as shown in (Figure 13 and 14).



**Figure (13):** Histological section of 4X magnification of control group at 28 days. The selected areas show formation of new compact bone closing defect.



**Figure (14):** Histological section of 4X of BLUE BONE material group at 28 days. The arrows show compact bone closing the defect.

**3.Statistical analysis:** all statistical results represented mean  $\pm$  standard error of mean, the small letter refers the compression within groups where change in the small letters means that there was a significant statistical difference while capital letters represent comparison

between groups where change in the capital letters mean statistical significant difference.

**1. Radiographic results:** Statistical analysis of radiographic results showed that there was statistically significant difference between defect filled with BLUE BONE material and control defect as shown in (Table 1).

**Table (1):** Statistical analysis of radiographical results, represented as mean ± standard error of mean at significant level 0.05.

	<b>3D</b> Mean ± SE	<b>1W</b> Mean ± SE	<b>2W</b> Mean ± SE	<b>4W</b> Mean ± SE
<b>CONTROL</b>	90.8±3.2 a A	137±6.5 b A	179.6±12.9 c A	181.6±9.8 c A
<b>Blue Bone</b>	112.4±7.3 a B	159.2±5.1 b B	201.2± 9.6 c B	209.2±10.7 c A

Small letters refer to comparison within group, their changes reflect statistically significant difference.  
Capital letters refer to comparison between groups their change reflect statistically significant difference.

**2. Newly formed bone below** and statistically there was significant difference  
Throughout the periods of study, the BLUE as shown in (Table 2).  
BONE material showed greater bone formation

**Table (2):** Statistical analysis of new formed bone area represented as mean ± standard error of mean at significant level 0.05.

	<b>3 Day</b>	<b>7 Day</b>	<b>14 Day</b>	<b>28 Day</b>
<b>Control</b>	0.00 a A	28559.38±1679.3 A	111486.82±1818.6 b c A	231346.76±4356.6 d A
<b>BLUE BONE</b>	33187.66±2834.0 a C	141797.766 ± 887.96 bB	400577.04±14045.7 c B	606911.72±6197.6 dB

Small letters refer to comparison within group, their changes reflect statistically significant difference.  
Capital letters refer to comparison between groups their change reflect statistically significant difference.

**3. Osteoblast numbers found:** group declined dramatically indicating that  
The osteoblast numbers at treated defect were most osteoblast changed to osteocyte. There  
greater than the numbers in control defect until was statistically significant difference between  
28 days. The number of osteoblasts at treated groups as shown in (Table 3).

**Table (3):** Statistical analysis of numbers of osteoblast represented as mean ± standard error of mean at significant level 0.05.

	<b>3 Day</b>	<b>7 Day</b>	<b>14 Day</b>	<b>28 Day</b>
<b>Control</b>	0.00 a A	11±0.7 b A	23.4±1.2 c A	16.8±0.9 d A
<b>BLUE BONE</b>	12.6±0.7 a B	26.6±1.08 b B	41.5±1.06 c B	10.8±0.8 a B

Small letters refer to comparison within group, their changes reflect statistically significant difference.  
Capital letters refer to comparison between groups their change reflect statistically significant difference.

**4. Osteoclast number found**

The osteoclasts numbers at treated defect were greater than the numbers in control defect until 28 days the number of osteoclast at treated

group declined dramatically indicating that most osteoclast finished their function. There was statistically significant difference between groups as shown in (Table 4).

**Table (4):** Statistical analysis of osteoclast number represented as mean ±standard error of mean at significant level 0.05.

	3 Day	7 Day	14 Day	28 Day
<b>COTROL</b>	0.00 a A	2.2±0.3 b A	3.4±0.4 c A	1.8±0.1 b A
<b>Blue bone</b>	1.8±0.2 a B	5.5±0.7 b C	5.3±0.8 b B	0.7±0.1 a B

Small letters refer to comparison within group, their changes reflect statistically significant difference. Capital letters refer to comparison between groups their change reflect statistically significant difference.

**DISCUSSION**

Radiographic results revealed higher radioopacity at treated group due to greater bone formation and higher mineralization so these results agreed with Chen *et al* 2017. The current study displayed enhancement of bone regeneration by using BLUE BONE material by osteoconductive properties of material as the biomaterial resorb free calcium and phosphate ions released and the change in the ions concentration stimulate formation and differentiation of osteoblast and eventually bone formation <sup>(12)</sup>. Our results agreed with Puttini *et al.* (2019) <sup>(13)</sup> who found greater bone formation by using BCP. The treated group showed greater cellular activity by presenting

higher numbers of osteoblast and osteoclast and this high numbers of cells at treated group may be due to porosities of material that permits diffusion of cells, angiogenesis and nutrients transportation. These porosities act as channels for migration of cells and formation of new blood vessels and this events founded by Ebrahimi *et al* (2014) <sup>(14)</sup> who found that BCP stimulate cellular accumulation. The inflammatory response at the defect treated with blue bone was lesser than that seen in control group which showed sever inflammatory response and this may be due to anti-inflammatory action of both hydroxyapatite and tricalcium phosphate. These results agreed with Sadowska *et al.* 2019 <sup>(15)</sup>

who found lesser inflammatory response to BCP. In conclusion, BLUE BONE material accelerates bone formation and show high potential capacity of bone regeneration.

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