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Research Article

Evaluation of Osseous Regeneration Using Autogenous Bone Graft, Biphasic Calcium Phosphate and Biphasic Calcium Phosphate Combined With Hyaluronic Acid in Critical Sized Rabbit Femoral Defects

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Keywords: *Bone Regeneration; Bone Substitutes; Autogenous Bone Graft; Biphasic Calcium Phosphate; Beta-Tricalcium Phosphate; Hyaluronic Acid*

Abstract

Objective

To compare bone regeneration in critical sized femoral defects in a rabbit model after the application of autogenous bone graft, biphasic calcium phosphate bone graft and biphasic calcium phosphate bone graft in adjunct to hyaluronic acid gel.

Materials and Methods

Six white New Zealand rabbits about 3-6 months of age were included in this study. Three identical bony defects were created in each femur. Defects are divided into three groups and filled with one of

the materials used in this study. Group I: Autogenous bone graft. Group II: Biphasic calcium phosphate (BCP) bone graft and Group III: Hyaluronic acid (HA) gel and BCP. Bone regeneration has been evaluated at 6 and 8 weeks after surgery by means of histological and histomorphometric investigations.

Results

No operative or postoperative complications were encountered. All materials used in this study produced favorable response and bone regeneration according to histological and histomorphometric examinations. Autogenous bone graft induced the highest percentage of new bone formation among all study groups at 6 and 8 weeks after surgery. Histomorphometric analysis presented a regenerated bone mean percentage of 43.091±2.229, 31.169±7.265 and 37.819±8.977 after 6 weeks of surgery in autogenous bone graft group, biphasic calcium phosphate group and biphasic calcium phosphate combined with hyaluronic acid group respectively. A statistically significant difference was found among the three groups.

The mean percentage of new bone formation at 8 weeks of surgery was 69.027±1.782, 49.860±2.188 and 59.518±7.298 in autogenous bone graft group, biphasic calcium phosphate group and biphasic calcium phosphate combined with hyaluronic acid group respectively. Comparing the three studied groups showed a statistically significant difference. Percentage of new bone formation in autogenous bone graft group was statistically significantly greater than that of biphasic calcium phosphate group at 6 and 8 weeks after surgery. The percentage of new bone formation at 8 weeks after surgery was statistically significantly higher than that recorded at 6 weeks after surgery in the three studied groups.

Conclusion

Based on the results of this study, autogenous bone graft, biphasic calcium phosphate and biphasic calcium phosphate combined with hyaluronic acid can induce bone formation in a critical sized rabbit femoral defect. However, the addition of hyaluronic acid to biphasic calcium phosphate enhances new bone formation.

Introduction

Autogenous bone grafts are considered the gold standard for bone induction as they preserve the viability of cells and do not induce any immunological response. These grafts contain live osteoblasts and osteoprogenitor stem cells and heal by osteogenesis. They constitute all the three components for tissue engineering, which are, scaffold, cells, and signaling molecules. With adequate vascularization, osteoprogenitor cells proliferate and bridge the gap between the bone graft and the recipient bone and form the first deposits of new bone [1].

Autogenous bone grafts were most commonly used for grafting, but the availability of the donor site and the inadequate quantity of the graft material caused limitations when these grafts were used. Allografts and xenografts presented a solution to these problems. However, there is a question about immunogenicity and disease transfer. Therefore, alloplastic materials were introduced which are synthetic, biocompatible, inorganic bone

graft substitutes, easily available, eliminate the need of a donor site, and carry no risk for disease transmission [2].

Calcium phosphate ceramics such as beta tricalcium phosphate (β-TCP) and hydroxyapatite have been widely used as autogenous bone graft substitutes due to their similarity to the mineral phase of natural bone, lack of immunogenic reactions, good biocompatibility, bioactivity, and osteoconductivity [3,4].

Biphasic calcium phosphates are compounds of β -TCP and hydroxyapatite [5]. Blending of β -TCP with hydroxyapatite made it possible to control the resorbability of the material and at the same time, it maintains its osteoconductive property. The use of a ratio of 40% β-TCP and 60% hydroxyapatite allows better control of the bioabsorbable capability of the graft material resulting in faster new bone formation. The most important feature of BCP ceramic is its ability to form a strong direct bond with the host bone, which results in a strong interface [2]. In addition, it shows high biocompatibility, osteoconductive properties and provides stability of the defect volume due to good integration with tissues [6].

The addition of hydroxyapatite to β-tricalcium phosphate provides optimal dissolution and good bioactivity, cell attachment, proliferation, and differentiation for new bone regeneration [7]. The stability of hydroxyapatite acts to maintain the augmented space, while β-TCP promotes bone formation within that space. This balance between resorption and solubilization guarantees the stability of the biomaterial while promoting new bone ingrowth [8].

More recently, studies have shown that BCP can be osteoinductive. The intrinsic osteoinductive properties of BCP appear to be related to its chemical composition and architectural features, such as the surface geometry, topography, pore size, and porosity, which permit free circulation of fluids and cells. In the field of tissue engineering, they represent promising scaffolds capable of carrying and inducing the differentiation of stem cells. These characteristics associated with the cost, effectiveness, unlimited supply and absence of disease transmission make them a viable alternative to autografts and allografts [9-12].

Hyaluronic acid is a naturally occurring linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, and other tissues. It has numerous physiological and structural functions, which include cellular and extracellular interactions, interactions with growth factors and tissue lubrication [13]. Recently, it has been proven that it improves early osteogenic differentiation *in vitro* [14].

Hyaluronic acid is one of the most hygroscopic molecules known in nature [15]. It suppresses tissue breakdown activating metalloproteinase inhibitors [16]. It stimulates clot formation [17], increases osteogenesis [18], and does not interfere in the calcification nodules during bone formation [19]. Furthermore, it facilitates cell migration and differentiation during tissue formation and repair of both soft and hard tissues [20]. It accelerates bone regeneration by chemotaxis, proliferation and differentiation of mesenchymal cells. It also shares bone induction properties with osteogenic substances such as bone morphogenetic protein-2 [21].

Since histological evaluation remains the only reliable method to determine the efficacy of regenerative therapy [22]. Therefore, the present study was employed in an attempt to evaluate the healing of surgically created bony defects in rabbit's femur after implantation of autogenous bone graft, biphasic calcium phosphate or biphasic calcium phosphate combined with hyaluronic acid both histologically and histomorphometrically.

Materials and Methods

Animals

Six white New Zealand rabbits were included in this study, about 3-6 months of age and weigh about 1-1.25kg. All animals were submitted to surgery in both femurs under general anesthesia.

Materials

Autogenous bone graft, Biphasic calcium phosphate bone graft (Guidor easy-graft CRYSTAL)* and hyaluronic acid gel (hyaDENT BG)**

Autogenous bone graft was retrieved by trephine bur and it was cut into small pieces by using hand instruments.

Guidor easy-graft CRYSTAL is a biphasic synthetic, in situ hardening bone graft substitute composed of 60% hydroxyapatite and 40% β-TCP [23]. Biphasic calcium phosphate granules are coated with a thin layer of resorbable polylactic and polyglycolic acid (PLGA). Just before application, the granules were mixed with N-methyl-2-pyrrolidone (NMP) biolinker, this hardens upon contact with blood forming a stable porous scaffold [24,25].

Hyaluronic acid gel, hyaDENT BG is composed of a mixture of cross-linked (1.6%) and natural (0.2%) hyaluronic acid. The formulation hyaDENT BG is a sterile, transparent and highly viscous gel obtained by crosslinking special hyaluronic acid of non-animal origin. Two to three weeks after the application, the gel is completely reabsorbed.

Experimental Surgical Procedure [26]:

The animals were anaesthetized using an intramuscular injection of Ketamine hydrochloride 30mg/kg body weight and Xylazine 5mg/kg body weight. The area over each femur was shaved and disinfected with an iodine solution (figure 1). The skin over the femoral bone was incised, reflected, and the superficial fasciae, muscle tissue and deep fasciae were also incised bluntly then, the periosteum was incised and the muscle was dissected bluntly by a periosteal elevator to expose the femur (figure 2). Three identical bony defects were created down to the bone marrow using a trephine drill under constant normal saline irrigation with a width of 3mm and depth of 4mm (figures 3 & 4). The wound site was irrigated with normal saline solution to remove any debris or foreign particles. The defects were filled with one of the materials used in this study. In group I, defects were filled with autogenous bone graft. In group II, defects were filled with Guidor easy-graft CRYSTAL. In group III, defects were filled with Guidor easy-graft CRYSTAL and hyaDENT BG (figures 5-7). Tissues were carefully repositioned and sutured (figure 8). Disinfection of the wound with Iodine solution was performed after suturing. Sutures were removed after 7 days of surgery. Postoperative care consists of administration of 0.1ml of ketoprofen daily for pain control and as antibiotic prophylaxis therapy, 0.6 Norfloxacin was administered subcutaneously for 3 days after surgery. After 6 weeks of surgery, three rabbits were sacrificed, the other three rabbits were sacrificed after 8 weeks of surgery and bone formation in the defects was evaluated both histologically and histomorphometrically.

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**Bioscience GmbH, Germany

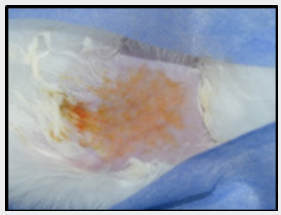

Figure 1: *The skin over the operation field is shaved and disinfected with 10% Iodine solution*

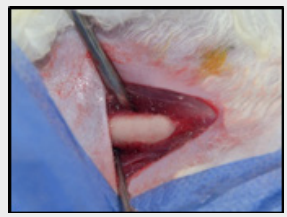

Figure 2: *Incisions though the skin and periosteum and lateral reflection of femoral muscles to expose the femoral bone was performed*

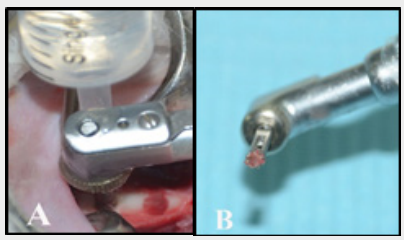

Figure 3A: *Creation of three identical bony defects using trephine bur in a vertical direction with a low speed handpiece and physiologic saline solution irrigation*
Figure 3B: *Harvesting autogenous bone graft*

Figure 4: *Three identical bony defects were created*

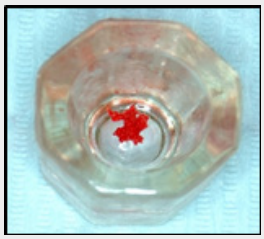

Figure 5: *Preparation of autogenous bone graft*

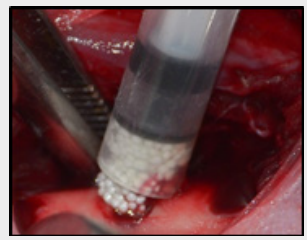

Figure 6: *Application of Guidor easy-graft CRYSTAL in the defect with the applicator syringe*

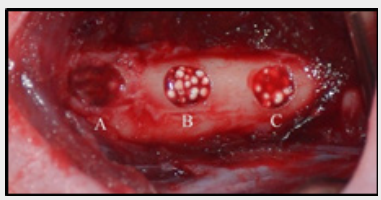

Figure 7: *Defects received A- Autogenous bone graft , B- Biphasic calcium phosphate, C- Biphasic calcium phosphate combined with hyaluronic acid gel*

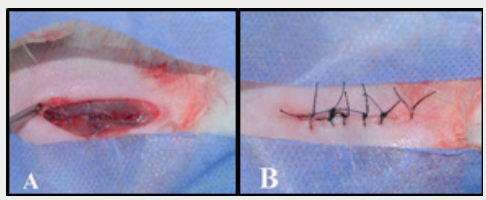

Figure 8A: *Suturing of deep fascia using 4-0 vicryl suture*
Figure 8B: *Suturing of the skin using 4-0 silk suture*

Histological Evaluation

Tissue samples were fixed in 10% neutral phosphate buffered formalin solution and decalcified in 10% Ethylenediaminetetraacetic acid (EDTA) solution containing 5% sodium sulfide until demineralization occurs. The bone specimens were dehydrated in alcohol and embedded in paraffin wax. Histological sections with a thickness of 4μm were cut and stained with haematoxylin and eosin (H&E) stain.

Histomorphometric Analysis

Bone histomorphometry was performed on IBM compatible computer using Image J software. From each slide, 10 arbitrarily selected microscopic fields with the same magnification were analyzed and the area of newly formed bone was calculated in relation to the total bone area found in the digitalized histological photos.

Statistical Methodology

Kolmogorov-Smirnov test of normality revealed no significance in the distribution of the variables, so the parametric statistics was adopted. Data were described using minimum, maximum, mean, standard deviation and 95% CI (confidence interval) of the mean. Comparisons were carried out between two studied independent normally distributed variables using independent sample t test. When Levene's test for equality of variances is significant, Welch's t-test is used. One-way Analysis Of Variance test was used. Post-hoc multiple comparisons was done using Games-Howell method. An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80% when calculating post-hoc power analysis.

Results

In this study, all rabbits completed the experimental period without any complications. The recovery after surgery was uneventful for all animals. The results of this study demonstrated that the tested materials supported satisfactory new bone formation leading to a total calcified tissue. In the histological sections, there were no signs of a cellular inflammatory reaction or infection around the used materials or the surrounding bony structure in any of the histological sections.

At 6 weeks after surgery, the autogenous bone graft treated defects showed greater bone formation throughout the bony defect area compared to the other two groups with the formation of anastomosing small, numerous and thin woven bony trabeculae with highly cellular marrow spaces. New bone was blending with the remaining graft particles present with complete bridging of the defect (figures 9-11). In group II and III, there was a significant amount of new bone tissue on the outer surface and inside the granules of the biphasic calcium phosphate product. The BCP granules appeared circular with different sizes. Osteoblast cells were observed at the surface of the bone graft substitute particles. Therefore, the graft granules were in contact with active osteoblasts forming new woven bone and demonstrating persistent osteogenesis. Active cellular resorption of the BCP particles was not detected. Newely formed bony tissue was found between the granules of the bone graft in both groups (figures 15-17, 21-23).

After 8 weeks of surgery, group I showed the highest amount of new bone formation with complete closure of the defect. However, residual autograft material could be detected and portions of autogenous bone graft were still present in defects augmented with autogenous bone grafts (figures 12-14). Group II and III showed

numerous anastomosing bony trabeculae surrounding the granules of easy-graft CRYSTAL. New bony regenerate was observed within resorbed regions of the allograft and between its particles. Both groups showed large amount of new bone formation within the defects which were nearly fully closed. Some residual particles of the bone graft substitute were still present in the defects (figures 18-20, 24-26).

Histological Results

Group I: *Autogenous Bone Graft*

After 6 weeks of surgery	After 8 weeks of surgery
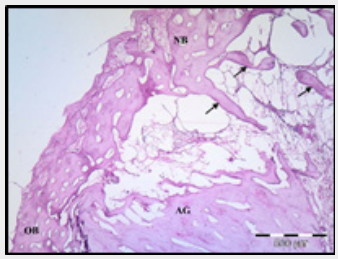	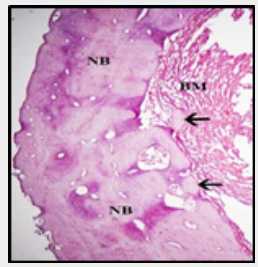
Figure 9: *Photomicrographs of group I after 6 weeks of surgery showing the autogenous bone graft (AG) with new bone formation (NB) in the form of small, numerous and thin woven bony trabeculae (black arrows) (H&E ×40).*	***Figure 12:*** *Photomicrograph of group I after 8 weeks of surgery showing new bone formation (NB) blending with the remaining portions of the autogenous bone graft particles (black arrows) which is noted within the bone marrow (BM) with a complete bony union across the defect (H&E ×40)*
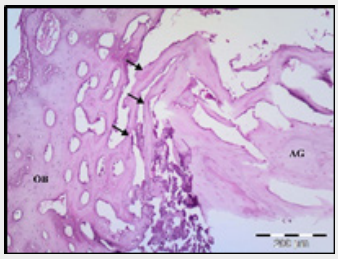	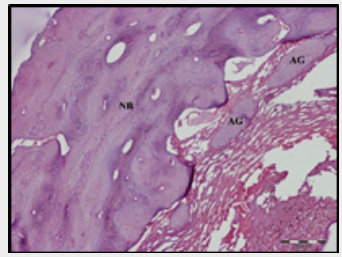
Figure 10: *Photomicrograph showing the autogenous bone graft (AG) blending with the original bone (OB) by forming bony spicules (black arrows) (H&E ×100)*	***Figure 13:*** *Photomicrograph showing that the defect is fully replaced by new bone (NB). There are still some remaining residual autogenous bone graft particles (AG) (H&E ×100)*
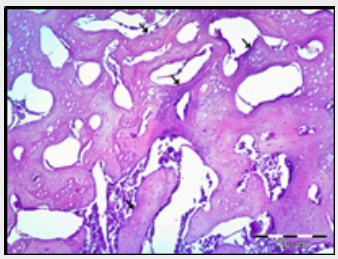	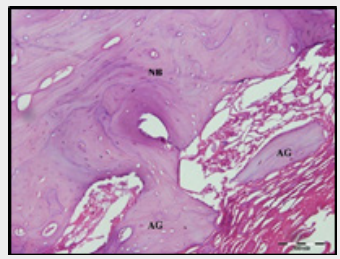
Figure 11: *Photomicrograph showing newly formed, numerous, thin, woven bony trabeculae (black arrows) in the bony defect area (H&E ×200)*	***Figure 14:*** *Residual autogenous bone graft material (AG) could be distinguished blending with the newly formed bone (NB) (H&E ×200)*

Group II: *Biphasic calcium phosphate*

After 6 weeks of surgery	After 8 weeks of surgery
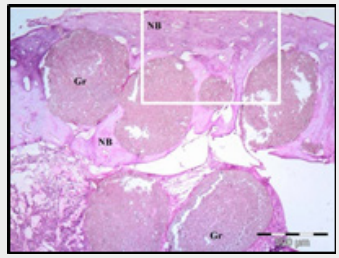	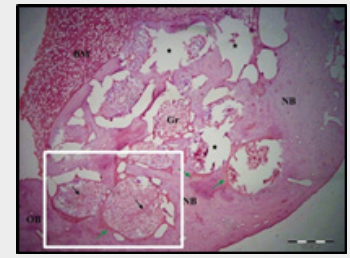
Figure 15: *Photomicrograph of group II after 6 weeks of surgery showing new bone formation (NB) surrounding the graft material particles (Gr) and closing the defect (H&E ×40)*	***Figure 18:*** *Photomicrograph of group II after 8 weeks of surgery showing newly formed bone (NB) surrounding the residual graft material (Gr) and closing the defect with complete union with the original bone (OB) with inegration of bone inside the graft granules (black arrows) with some resorption of the graft particles (black stars). Osteoid tissue was observed around the graft particles (green arrows) (H & E ×40)*
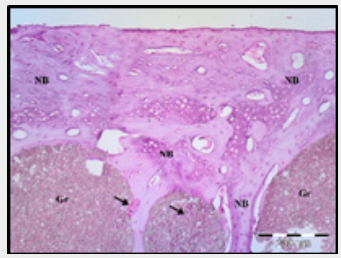	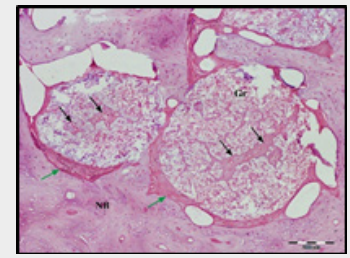
Figure 16: *Higher magnification of the boxed region in the previous image showing the newly formed bone (NB) above and in between the graft material particles (Gr) in the defect with integration of osteoid tissue within and on the periphery of the graft particles (black arrows) (H&E ×100)*	***Figure 19:*** *High power of the boxed region in the previous photomicrograph showing that easy-graft crystal granule (Gr) is penetrated by bone (black arrows). Osteoid tissue formation was observed around the graft particles (green arrows) (H&E ×100)*
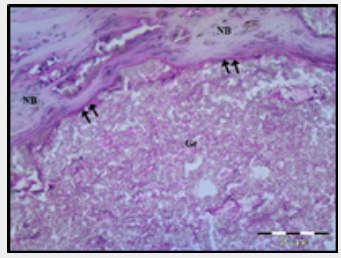	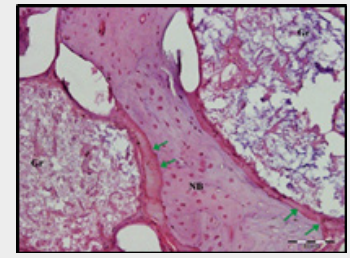
Figure 17: *Photomicrograph with higher magnification showing the integration of easy-graft CRYSTAL particle (Gr) into newly formed bone (NB) and tight contact (black arrows) was observed between the graft particle and new bone (H&E ×400)*	***Figure 20:*** *Photomicrograph showing the interface between graft particles (Gr) and new bone (NB) with osteoblasts forming osteoid tissue (green arrows) and new bone on the surface of easy-graft CRYSTAL particles (Gr) (H&E ×200)*

Group III: *Biphasic calcium phosphate and hyaluronic acid*

After 6 weeks of surgery	After 8 weeks of surgery
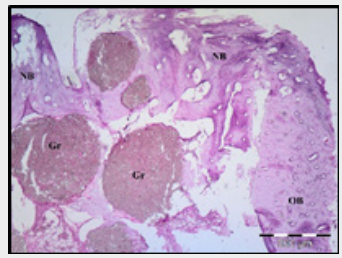	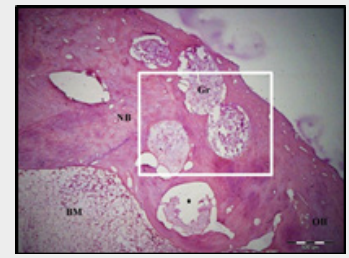
Figure 21: *Photomicrograph of group III after 6 weeks of surgery showing graft material particles (Gr) with new bone formation (NB) blending with the original bone (OB) and bridging the gap between the defect margins and nearly closing the defect (H&E ×40)*	***Figure 24:*** *photomicrograph of group III after 8 weeks of surgery showing graft particles (Gr) completely integrated within newly formed bone (NB) next to the bone marrow (BM). Some resorptin of the bone graft is noticed (black star).) (H&E ×40)*
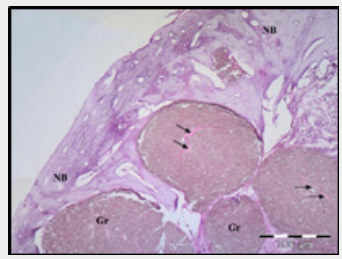	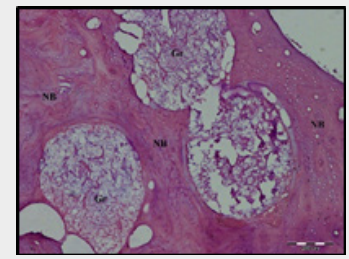
Figure 22: *Another photomicrograph showing that osteoid tissue (black arrows) is integrated within the particles of the graft material (Gr) which is surrounded by newly formed bone (NB) throughout the defect area. (H&E ×40)*	***Figure 25:*** *High power of the boxed region in the previous photomicrograph showing bone graft particles (Gr) completely embeded in newly formed bone (NB) (H&E ×100)*
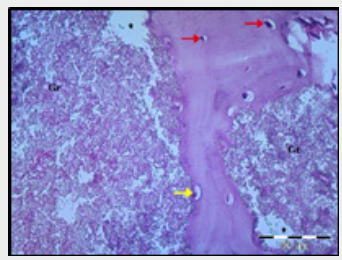	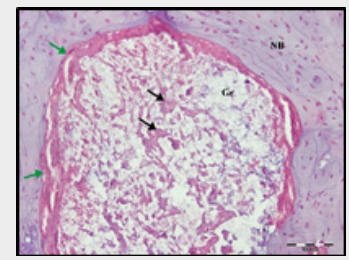
Figure 23: *Photomicrograph showing that the graft granules (Gr) are surrounded by newly formed bone. Osteoblasts (yellow arrows) were found directly on the periphery of the graft granules. Osteocytes (red arrows) were noted within the newly formed bone tissue with some areas of resorption of the osteoplastic material (black stars) (H&E ×400)*	***Figure 26:*** *Intragranular calcification (black arrows) and bone deposition surrounding the granules of the alloplastic material (NB) with osteoid tissue formation around the bone graft granules (green arrows) (H&E ×200)*

Histomorphometric Results (table 1):

Histomorphometric analysis presented a regenerated bone mean percentage of 43.091±2.229, 31.169±7.265 and 37.819±8.977 after 6 weeks of surgery in autogenous bone graft group, biphasic calcium phosphate group and biphasic calcium phosphate combined with hyaluronic acid group respectively. A statistically significant difference was found among the three groups (F=3.870, p=0.035). Post-hoc pairwise comparison showed that percentage of new bone formation in autogenous bone graft group was statistically significantly higher than that of biphasic calcium phosphate group (p=0.041).

The mean percentage of new bone formation at 8 weeks of surgery was 69.027±1.782, 49.860±2.188 and 59.518±7.298 in autogenous bone graft group, biphasic calcium phosphate group and biphasic calcium phosphate combined with hyaluronic acid group respectively. A statistically significant difference was found among the three groups (F=22.503, p=0.000). Post-hoc pairwise comparison showed that percentage of new bone formation in autogenous bone graft group was statistically significantly greater than that of biphasic calcium phosphate group (p=0.000).

Percentage of new bone formation at 8 weeks after surgery was statistically significantly higher than that recorded at 6 weeks in autogenous bone graft group (t=20.322, p=0.000), biphasic calcium phosphate group (t=5.508, p=0.003) and biphasic calcium phosphate combined with hyaluronic acid group (t=4.194, p=0.003).

Table 1: *Comparison of the mean percentage of new bone formation between the three studied groups at 6 and 8 weeks after surgery*

	Group			Test of significance
	Autogenous Bone Graft	Biphasic Calcium Phosphate	Biphasic Calcium Phosphate + Hyaluronic Acid	
Area (6 weeks) (%)				
n Min-Max Mean ± SD 95% CI	5 40.352 - 45.959 43.091 ± 2.229 40.324 - 45.859	5 21.255 - 38.382 31.169 ± 7.265 22.148 - 40.189	5 27.702 - 47.877 37.819 ± 8.977 26.672 - 48.965	F=3.870 $p_{(W)}$=0.035*
Autogenous Bone Graft		Diff=11.922 p=0.041*	Diff=5.272 p=0.472 NS	
Biphasic Calcium Phosphate			Diff=-6.649 p=0.442 NS	
Area (8 weeks) (%)				
n Min-Max Mean ± SD 95% CI	5 66.938 - 70.602 69.027 ± 1.782 66.814 - 71.240	5 47.654 - 52.906 49.860 ± 2.188 47.143 - 52.577	5 51.018 - 65.891 59.518 ± 7.298 50.456 - 68.579	F=22.503 $p_{(W)}$=0.000*
Autogenous Bone Graft		Diff=19.167 p=0.000*	Diff=9.509 p=0.088 NS	
Biphasic Calcium Phosphate			Diff=-9.658 p=0.084 NS	
Comparison between 6 weeks and 8 weeks				
Test of significance	t=20.322 p=0.000*	t=5.508 $p_{(W)}$=0.003*	t=4.194 p=0.003*	

n: Sample size per group
Min-Max: Minimum to maximum
SD: Standard deviation
W: Welch correction for p value
Post-hoc multiple (pairwise) comparison by Games-Howell method

Discussion

Autogenous bone graft group showed the highest mean percentage of new bone formation at 6 and 8 weeks after surgery followed by biphasic calcium phosphate combined with hyaluronic acid group then, biphasic calcium phosphate group, this is explained by the fact that autogenous bone grafts have an osteoinductive and osteoconductive role and are considered to be the gold standard since there is a possibility to retain cell viability and graft revascularization. However, there is added operating time associated with their harvest [27,28].

It can also be explained by the fact that BCP undergoes partial resorption mainly due to β-tricalcium phosphate while, hydroxyapatite remains in the defect area for a prolonged period of time thus preventing atrophy and preserving the bone tissue volume [29,30]. It was found that, after the implantation of BCP into the bone defect, the β- TCP phase of the material undergoes resorption and replacement by the bone tissue during 6-7 months [31].

Percentage of new bone formation in the BCP group obtained in the present study after 6 weeks of surgery was greater than that obtained by Salamanca *et al.*, (2018) [32] as they reported 14.89% ± 9.18% new bone formation at 6 weeks after surgery with the use of BCP in rabbit clavarial critical size defects . Moreover, the percentage of new bone formation after 6 weeks using biphasic calcium phosphate in this study was more than that obtained by Schmidlin *et al.*, (2013) [5] using Guidor easy-graft CRYSTAL in rabbit calvarial defects model. They reported 20.16% ± 5.27% of new bone after 4 weeks of healing. This variance can be attributed to the difference in time interval after surgery in the two studies.

The mean percentage of newly formed bone at 6 and 8 weeks after surgery in biphasic calcium phosphate group and biphasic calcium phosphate combined with hyaluronic acid group is less than that in the autogenous bone graft group, this may be due to the low rate of resorption of the osteoplastic material because biphasic osteoplastic material undergoes resorption by β-tricalcium phosphate phase of the biomaterial, whereas hydroxyapatite does not resorb for a long time and keeps its occupied area [33].

Bone formation in this study is in accordance with the studies conducted by Ghanaati *et al.*,(2012) [34]; and Sager *et al.*, (2012) [35] in which they demonstrated a positive effect on osteogenesis of BCP with hydroxyapatite /β- tricalcium phosphate mixture with 60/40 wt % ratio.

Histological findings of this study also are in accordance with the results obtained by Korenkov (2016) [6] who reported that osteogenic cells were located on the surface and inside the granules of easy-graft CRYSTAL demonstrating the osteoconductive properties of the osteoplastic material and its good integration with the tissue specific structures of the regenerate.

To improve osteoconductive effects of biphasic calcium phosphate, they have been combined with various biocompatible materials, such as hyaluronic acid [36]. Hyaluronic acid is of special interest for bone substitute materials by providing additional regenerative features by modulation of wound healing, cellular migration, and angiogenesis via specific receptors such as the CD44 molecule [37]. One of the properties the cross-linked hyaluronic acid gel utilized in this study is its ability to facilitate new bone formation. Its cross-linked feature has been shown to improve the overall mechanical properties of the scaffold material, further stimulating osteogenesis [38].

This study demonstrated that the osteoplastic material biphasic calcium phosphate and the hyaluronic acid gel are characterized by high biocompatibility, as evidenced by the absence of any inflammatory reaction throughout the entire duration of the experiment. Hyaluronic acid gel when mixed with bone substitute material of any origin, leads to accelerated bone formation [21,39].

The results of the present study showed that the combination of hyaluronic acid with the bone substitute materials promoted more new bone formation than the bone substitute alone. Thus, this combination with bone

substitutes is opening the door to innovative therapeutic alternatives in the management of bony defects. This is in accordance with studies which proved that the cross-linked hyaluronic acid in the form of viscous gel allowed the hyaluronic acid to bind to the granules of the bone graft easily and also, demonstrated that hyaluronic acid is used to promote healing in both soft tissue and bone regeneration [40,41].

The findings of the current study are also in accordance with an *in vivo* study by Ghanaati *et al* (2011) [42] in which they confirmed that, a bone substitute containing β-TCP granules combined with a liquid phase of hyaluronic acid triggers implant bed vascularization as an important factor of bone tissue regeneration. These results provide an evidence that HA combined with BCP is beneficial for tissue engineering [43], which is in agreement with previous reports suggesting that bone grafts combined with HA enhance bone growth and mineralization [15,44].

The improved bone regenerative capacity with the addition of HA in the current study is in agreement with the study performed in 2014 by Nguyen and Lee [44] who prepared a scaffold by loading hyaluronic acid hydrogel into BCP ceramic. After a series of animal studies, they suggested that this novel bone substitute exhibited rapid new bone formation and a high rate of collagen mineralization. Overall, biphasic calcium phosphate mixed with hyaluronic acid exhibits a great promise for use in stimulating new bone formation.

The results of the present study were in contrary to the results obtained by Yip *et al.*,(2015) [45] who supposed that the poly lactide-co-glycolide acid (PLGA) layer or the N-methyl-2-pyrrolidone (NMP) containing plasticizer, which are required to provide the unique properties of the alloplastic material, might jeopardize the bone induction and integration of the material. However, the present study showed that new bone formation was found in close contact with the bone graft granule as well as within the graft granules. This is in accordance with the study performed by Wildburger *et al.*, (2017) [46] who assessed the influence of the in situ hardening properties of a biphasic calcium phosphate bone graft substitute compared to a particulate bone graft substitute with the same biphasic core-granule composition without in situ hardening properties on sinus floor augmentation. The results showed that the bone growth rate for PLGA-NMP-BCP was higher than the growth rate for BCP and they concluded that the PLGA coating did not influence the osseointegration of the calcium phosphate particles.

Conclusion

In light of the findings of the present study, it was demonstrated that all materials used in this study represented a promising method for osseous regeneration. However, autogenous bone graft produced greater percentage of bone fill followed by biphasic calcium phosphate combined with hyaluronic acid gel then, biphasic calcium phosphate.

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Conflicts of Interest

There are no conflicts of interest.

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