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BIOLOGICAL BEHAVIOR OF 60/40 BIPHASIC CALCIUM PHOSPHATE AND GROWTH HORMONE MIXTURE: IN VIVO STUDY IN DOGS

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ABSTRACT

Grafting materials are important for ridge preservation procedures after tooth extraction. Ridge preservation prime value is the volume preservation for the healing sockets, which is needed for different ridge augmentation procedures.

Objective: Determine the healing responses of (local growth hormone, and biphasic calcium phosphate mixture 60/40 in fresh extraction sockets of a canine model.

Materials and Methods: Six mongrel dogs included in this study; they were divided into 2 groups according to the time of sacrifice (4 and 8 weeks). Each of these groups includes three animals. Animals subjected to extraction of the fourth premolar bilaterally, The right side treated with a mixture of 4 IU (1.6 mg) Growth hormone and biphasic calcium phosphate (a composite of hydroxyapatite HA and bèta-tricalcium phosphate TCP 1 x 0.15 ml (450-650 μm) in a granular form, while the left side left as a control. The healing extraction sockets were evaluated by surface electron microscope (SEM).

Results: Scanning electron microscope examination showed homogenous new bone formation in both groups. Control group showed significant results as regard calcium phosphate ratio in the first month, while non-significant result was encountered in the second month between two groups.

Conclusion:. Single local dose of growth hormone mixed with 60/40 biphasic calcium phosphate did not improve calcium phosphate ratio.

KEYWORDS: Calcium phosphate ratio, surface electron microscope, ridge preservation, biphasic calcium phosphate and dogs.

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INTRODUCTION

Alveolar bone remodeling is a tooth-dependent process. Once the tooth extracted, trauma stimulates the activity of osteoclasts leading to alveolar bone resorption. Resultant clinical bone loss in height and width leads to essential problems for restorative and implant dentistry at the extraction site. The effect is magnified when multiple teeth were extracted in the area. [1- 5]

Preservation procedures using various bone substitutes are recommended. Synthetic bone grafts were preferable for their availability, and low risk for disease transmission. Basically, they are made of hydroxyapatite (HA) or tricalcium phosphate (TCP), they have different biodegradability. The rationale for the development of biphasic calcium phosphate bioceramics has been their similarity in composition to bone mineral phase and biodegradability, bioactivity and osteoconductivity. Moreover, biphasic calcium phosphate bioceramics (60 HA / 40 beta -TCP) has the advantage of adequate mechanical strength and reasonable resorption rate that allow new bone deposition [6-15]

Growth hormone (GH) local administration in a single dose at time of surgery is promising. It could regulate local bone metabolism by enhancing the proliferation of osteoblastic cell lines, and express bone morphogenetic proteins (BMP) especially in the early stages of healing. [2, 16- 18]

Modern computers availability had enabled the scanning electron microscope (SEM), which is important for the observation of surface topography. Moreover, it determines the chemical composition of a specimen by using energy dispersive x-ray analysis (EDX), having the advantages of minimum specimen preparation, relatively quick and nondestructive mean [19, 20].

This study used a combination of biphasic calcium phosphate 60/40 and growth hormone at the fresh extraction site for the purpose of early new bone formation and volume preservation.

MATERIALS AND METHODS

The experimental study was performed at the Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Suez Canal University. Six adult healthy male mongrel dogs with an average weight of 10-20 kg, comparable age (about 1year) included in this study. Animals divided into 2 groups according to the time of sacrifice (4 and 8 weeks). Each of these groups includes three animals.

All surgical procedures performed using injectable general anesthesia, each dog premedicated with intramuscular injection of Valpam (*Amoun Pharmaceutical Industries co. (APIC) Cairo, Egypt.)* in a dose of 0.5mg / Kg body weight 10-15 min. before operation. Induction and maintenance anesthesia carried out using IV administration of Sodium thiopental (*Egyptian International Pharmaceutical Industries co(EIPICO) 10 th of Ramadan city, EGYPT*) 2.5% solution 20-30 mg/kg body weight.

Injection of prophylactic antibiotic carried out using Flumox (*Egyptian International Pharmaceutical Industries co(EIPICO) 1O th of Ramadan city ,EGYPT.)* 500mg, 2ml IV every 24 hours for 5 days

Animals subjected to extraction of the fourth premolar bilaterally **(Fig.1A&B)**. The right side treated with a mixture of 4 IU (1.6 mg) Growth hormone (*Somatropin 4 IU Sedico Pharmaceutical Industries co,6 th October city.EGYPT)* and easy-graft- crystal *(Wagistrasse 23 - 8952 Schlieren/ Zurich-Switzerland*) 1 x 0.15 ml (450-650 μm) in a granular form, while the left side left as a control **(Fig.2A)**. Releasing incisions followed for primary wound closure using 4.0 vicryl sutures **(Fig.2B)**. Postoperative analgesic (*CATAFLAM ®NOVARTIS PHARMA Pharmaceutical industries co,CAIRO,EGYPT.*) 75mg / 2ml IM administrated once a day for 3 days. Plaque control with chlorohexidine (*ANTISEPTOL KAHIRA, Cairo, Egypt)* mouth lavage was used.

Fig. (1) A: Showing 4 th premolar tooth. B: Showing the extraction socket.

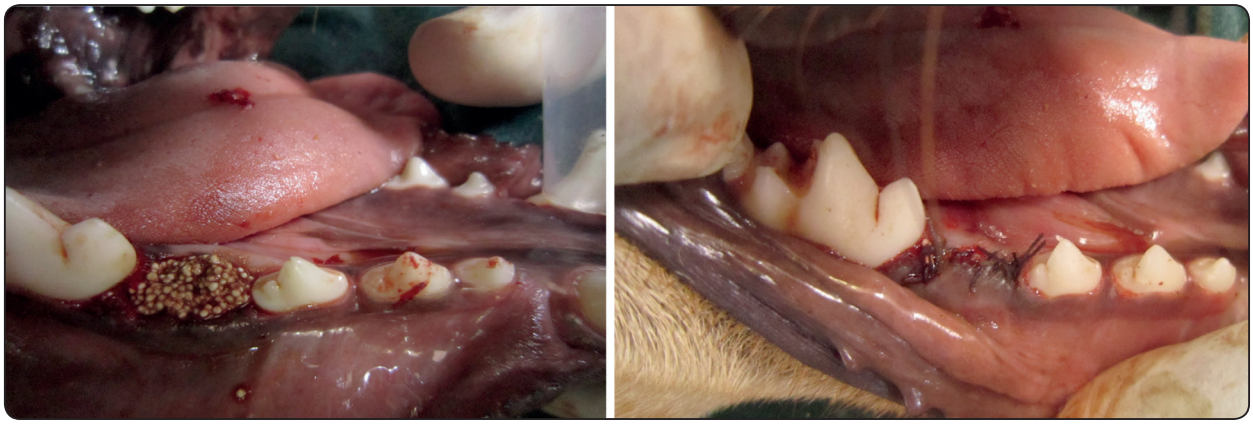

Fig. (2) A: Showing biphasic calcium phosphate granules in the socket. B: Showing primary closure.

Animal sacrifice performed by rapid IV injection of thiopental sodium overdose. The mandibles dissected, sectioned and put in neutral formalin 10%.

Histomorphometric analysis

1-SEM analysis

Bone blocks were removed from formalin and gently washed before analysis under distilled water, then accurately dehydrated. The studied samples were undersized (1½ x1 cm) to match the sensors inside the device **(Fig.3 A)**. The assessment done for the new bone formed midway in the socket **(Fig.3 B)** and the bone proper around socket for each sample.

The scanning voltage was 80 Kv, and the exposure time was 1second. A total of 1600 projections were acquired, while the specimen was rotated about 360°. The pixel size was 22.4814 μm. Then, the visualization and quantification of the newly formed bone were fulfilled on Avizo Fire (Visualization Sciences Group, FEI Corp., OR) image analysis software.

Statistical analysis

Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, and USA) Statistics Version 24 for Windows. Data presented as Mean, standard deviation (SD. Data explored for normality using Shapiro-Wilk tests. Data for chemical analysis showed nonparametric distribution, so Mann-Whitney test used to compare different groups. Wilcoxon Signed rank test used to compare between different follow-up periods. The significance level was set at $P \leq 0.05$.

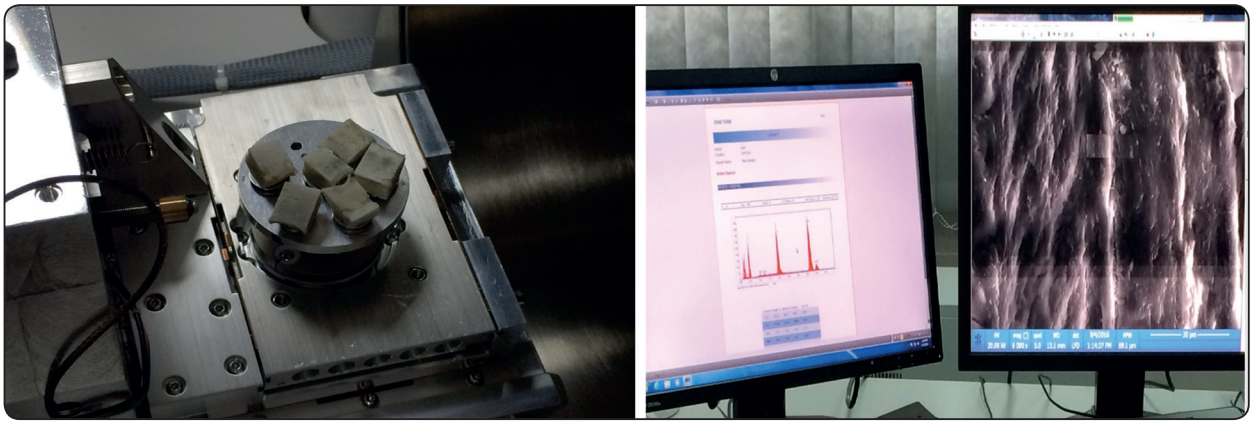

Fig. (3) A: Specimen inside the SEM sensor. B; Software analysis for each specimen

RESULTS:

1. Scanning Electron Microscope Examination at one month and 2 months respectively:

The scanning electron microscope examination showed homogenous intimate contact new bone formation in both groups **(Figures from 4 -7).**

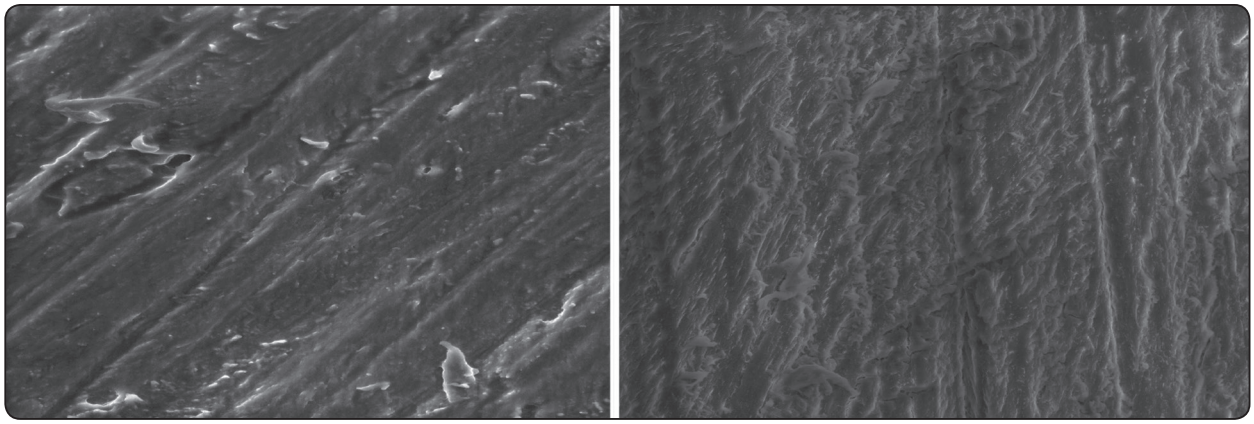

Fig. (4) Scanning electron microscope showed: (a) Control bone surface at 1 month (b) bone surface treated with (HA/TCP) -GH composite at 1 month (X 50)

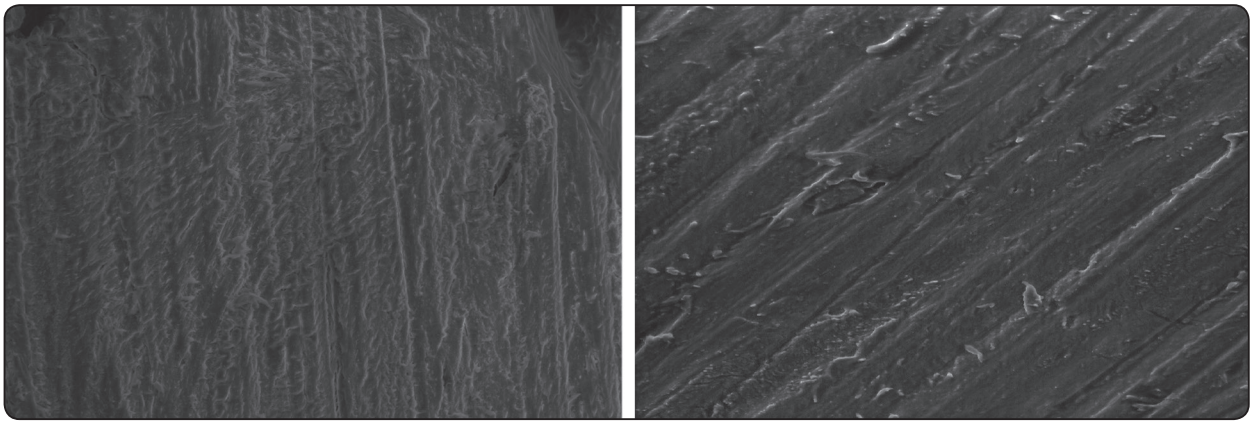

Fig. (5) Scanning electron microscope showed : (a) Control bone surface at 1 month (b) bone surface treated with (HA/TCP) -GH composite at 1 month(X 100)

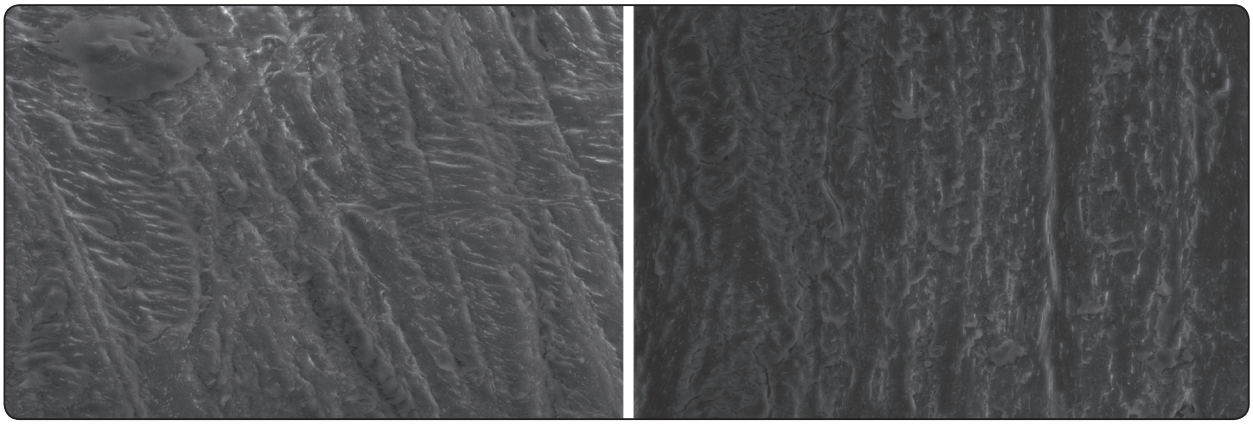

Fig. 6: Scanning electron microscope showed: (a) Control bone surface at 2 month (b) bone surface treated with (HA/TCP) -GH composite at 2 month (X 50)

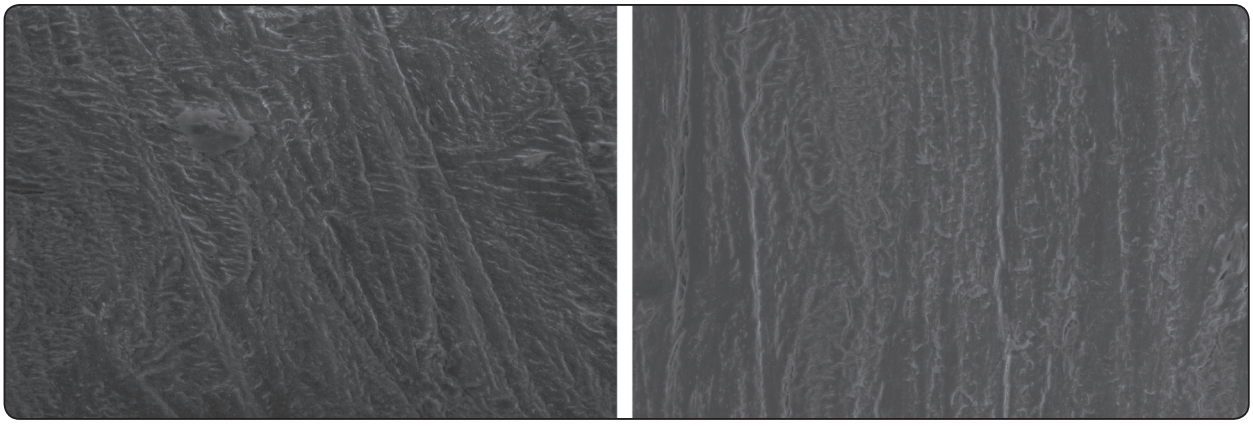

Fig. 7: Scanning electron microscope showed: (a) Control bone surface at 2 month (b) bone surface treated with (HA/TCP) -GH composite at 2 month (X 100)

2. Energy Dispersive Spectrometry

The energy dispersive spectrometry (EDS) analysis of the HA/TCP and GH composite showed chemical analysis composition in form of calcium (CA)/phosphate (P) ratio **(Fig. 8), and Tables 1**

I- Comparison between Ca/P ratio in control and test groups

At one month, a higher mean value was recorded in control group, with a significant difference (P=0.017), **(Table 1, Fig. 9)**

At two months, a higher mean value was recorded in test group, with no significant difference (P=0.78), **(Table 1, Fig. 9)**

II- Comparison between Ca/P ratio in bone proper and both groups

At one month, a higher mean value was recorded in bone proper, with an extremely significant difference (P<0.0001), **(Table 2, Fig. 9)**

At two months, a slightly higher mean value was recorded in test in comparison to original bone, with no significant difference (P=0.86, P=0.35 for control and test group respectively), **(Table 2, Fig. 9)**

TABLE (1): Comparison between Ca/P ratio in control and test groups

Groups	CA/P ratio means	P
1M Control	2.07±0.02	0.017*
1M Test	2.01±0.04	
2M Control	2.27±0.05	0.78^{ns}
2M Test	2.28±0.06	

Significance level P<0.05, *significant, ns=non-significant

TABLE (2) Comparison between Ca/P ratio in Bone proper, control and test groups (Unpaired t- test)

Groups	CA/P ratio	Bone proper	P
1M Control	2.07±0.02	2.3±0.05	<0.0001*
1M Test	2.01 ±0.04		<0.0001*
2M Control	2.27±0.05	2.22±0.12	0.86^{ns}
2M Test	2.28±0.06		0.35^{ns}

Significance level P<0.05, *significant, ns=non-significant

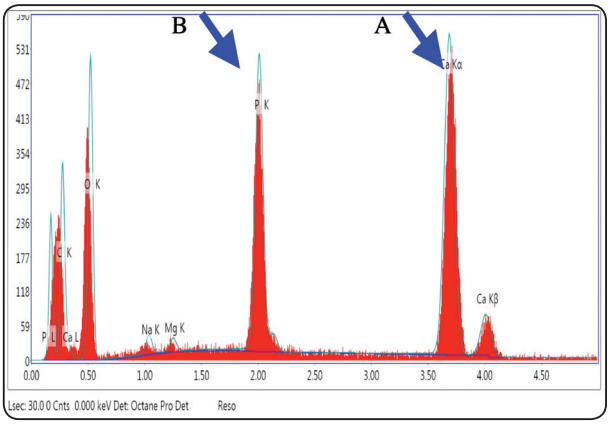

Fig. (8) EDX spectrum showing calcium(CA) and phosphate (P) heights

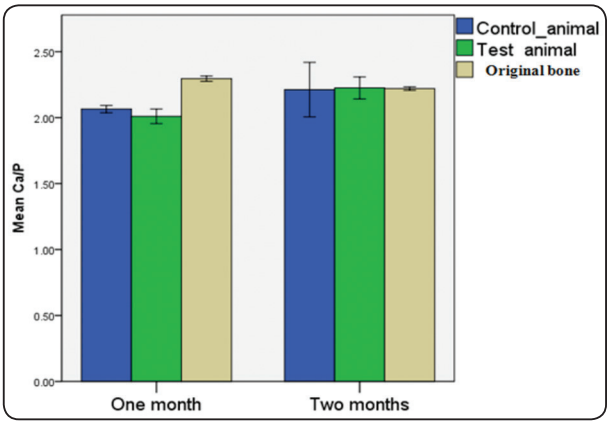

Fig. 9: Schematic representation for control, test and bone proper.

DISCUSSION

Calcium phosphate bioceramics application has been investigated in vitro and in vivo in various studies. It is a biocompatible material having osteoconductive properties which provide suitable environment for bone formation in vivo.[21 - 27]

Biphasic calcium phosphates (BCP) bioceramics granules determine the ideal balance between stable phase HA and resorbable phase β-TCP to control in vivo biodegradability. However, there is always a debate as regard the optimum composition ratio.[28-30]

Calcium and phosphate are the major mineral components in bone. Measuring the Ca/P ratio is a good index of bone quality. Relationship had been confirmed between bone loss and lowered CA/P ratio. Changes in CA/ P ratio is a good indicator for changes in the organic matrix of bone, specially collagen.

Various BCP composition ratios have been studied. Two ratios of HA/b-TCP (50/50 and 60/40) were under clinical trial in humans. Brodie et al. reported that collagen coating increases the strength of HA/TCP 50/50, whereas it weakens HA/TCP

25/75 and pure TCP discs invitro study. This may be related to increased TCP ratio. While Tanimoto Y et al 2009 proved that the ratio of 25/TCP 75 had the best cellular activity in vitro study. [35, 36]

The suggested ratio in this study was 60/40 ratio of (HA/ β-TCP) supposed to provide a balance a regard the strength during growth hormone addition as a source of collagen.

Therefore, development of organic-inorganic hybrid BCP scaffolds provides excellent possibilities for optimizing the conventional bone substitutes. [37] Ying et 2018, evaluated the osteoinductive property of the calcium phosphate bioceramics. He found that the synergistic effect of biphasic calcium phosphate ceramics on seeded macrophages will enhance directed migration of bone marrow-derived mesenchymal stem cells toward the ceramic surface. These stem cells will be stimulated to differentiate into vascular endothelial cells leading to the formation of new blood vessels enhancing new bone. [38]

Biphasic calcium phosphates have been used as a drug delivery systems and tissue engineering scaffolds. [39, 40] They were degraded into CA and phosphate those used for benefit of new bone formation. There crystals exhibit a surface interaction and physicochemical properties making them potential candidates for local delivery of various drugs as hormones, steroids, antibiotics and BMP-2. [41- 44] From all above, this study supposed" GH as a collagen source and as drug to be carried on top of BCP leads to osteoprogenitor cell differentiation with upregulation of osteoblast activity. [45- 47]

Ko-Ning et al 2016 studied the performance of ceramic composite composed of purified fibrillar collagen and HA/TCP in socket preservation in beagle dogs. At eight weeks there scanning electron microscope examination showed that HA/TCP granules were homogenously distributed with the collagen matrix. At the same time, energy dispersive spectrometry analysis of the HA/TCP collagen composite showed CA/P ratio equal to 1.83. [48] These results were consistent with our study as at two months scanning electron microscope examination showed homogenous new bone formation in both groups (test and control). The energy dispersive spectrometry (EDS) analysis of the HA/TCP/GH composite, showed chemical analysis composition of calcium CA/P ratio equal 2.28±0.06 at test group and 2.27±0.05 at control group. In 2014 Rander et al used histological and scanning electron microscopy (SEM) analysis to evaluate the bone integration of biphasic calcium phosphate BCP bioceramic in rats extraction sockets. There results showed that BCP 60/40 bone graft is a biocompatible material as histological results showed no foreign body reactions or any persistent inflammation. At 7 days, SEM microscopy noticed close contact between connective tissue and bioceramic particles. In 21 to 42 days, scanning electron microscopy analysis showed newly formed bone adhered to ceramic interface with no interposition of soft tissue.[49] This was also obtained by Kitsugi et al 1995 and Fujita et al 2014, who found direct connection between bioceramics and newly formed osteoid matrix. [50, 51]

In conclusion, biphasic calcium phosphate and growth hormone (most of its components were collagen) BCP/GH mixture, in one single dose at time of surgery did not make a difference as regard the rate of new bone deposition.

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