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# Facilitating osteogenesis of Hydroxyapatite granules by Autogenous bone marrow

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**Abstract**

The aim study to evaluate whether Hydroxyapatite granules alone or in combination with autogenous bone marrow can facilitate bone formation. Materials and Methods**:** (8) dogs of both sexes were used as a model in this experimental study ,they were divided into two groups , the control group in which ( 4) dogs were operated on in this group received Hydroxyapatite granules alone in defect created in the body of mandible (1cm) in diameter and experimental group in which (4)dogs were operated on in this group received Hydroxyapatite granules mixed with autogenous bone marrow implanted in the defect also created in the body of mandible (1cm) in diameter .Alkaline phosphatase enzyme (ALP) used as parameter to evaluated the degree of osteogenesis ,so blood sampling from femur vein was aspirated at 1st ,2nd ,3rd and 4th weeks respectivelly for biochemical analysis .Results :Statistical analysis showed that there is significant elevation of serum (ALP) in experimental group at day 15 and 21 comparing to control group .Conclusion: Hydroxyapatite with autogenous bone marrow than it is used alone. has great effect on facilitating of osteogenesis.

**Key words :hydroxyapatite,bone morphogenic protein,bone marrow.**

## Introduction

The outogenous bone graft are the basic material for bony defect ,but limitation in amount of such graft in the implantation of large bony defect , synthetic bone substitutes are used of various materials and sources[1].The basic requirements of such materials should be biocompatible with host tissue, able to simulate bone induction ,resorbable following implantation ,radiopaque ,inexpensive, sufficient porosity to allow bone conduction and growth[2].One of these materials is hydroxyapatite it is widely used as bone substitute in oral and maxillofacial surgery due to there osteoconductive properties, it has agreat potential of bone replacement materials because similarity to the crystals structures of inorganic matrix of bone and because of such potential properties , surgically it is used as alveolar ridge augmentation[3], in the treatment of periodontal osseous defect [4] and in sinus lifting procedure [5].Hydroxyapatite indicated in small and medium sized defect but in case of large bony defect and great tensile strength was needed ,this materials have to be supplemented with other materials like bone marrow in which bone morphogenic protein is present (BMP) [6],such recombination or fusion ,the result is equal to that of outogenous cancellous bone [7] . This combination mixed as the paste and put in area of missing bone to facilitating new bone growth . Another

combination of hydroxyapatite is with collagen composite (COL-HA) has been reported and such combination has the potential mimicking and replacing skeletal bone [8].At the histological level active osteoblast together with bone formation in contact with the porous surface of hydroxyapatite was observed with no cartilage formation after 6 months and can not be identified from old bone grossly [9].Experimental study on rats using (BMP) in the treatment of large bony defect of the mandible[10] the results indicated significantly facilitating bone deposition comparing to control.Other study on rabbits using porous ceramic hydroxyapatite with outogenous bone marrow ,the authors indicated highly significant elevation of serum ALP ( active osteogenesis) following 3 weeks of implantation in comparing to control[11]. Another study on rabbit containing hydroxyapatite and tricalicum phosphate (HA/TCP) with human morphogenic protein rHBMP [12], the immobilized rHBMP could lead to an enhancement of bone growth and replacement of artificial hydroxyapatite by endogenous bone ,this result showed that BMP2 can chemically immobilized on porous hydroxyapatite wafers[13]. A mixture of hydroxyapatite with collagen composite dropped with Zn++ cyto compatibility provide rapid osteogenesis as that observed and investigated by ALP[14].The ability of marrow derived osteoprogenitor cells to promote repair treated of large bony defect (4-7 cm)tested in sheep model should that bone formation was formed to occur both within the internal macro porous spaces and around the hydroxyapatite [15].

## Materials and Methods

The number of dogs operated on were (8) aged (3-5) years apparent healthy.Animals kept in cages received normal diet for 4 days before the day of operation.Aneasthesia of Animal**,**General aneasthesia was used by Ketamine HCL(15mg/kg)with Xylazine (5mg/kg)both were given IM.
Surgical procedure**:** The surgical procedure include two parts as follow:

**Part I** , (Mandibular surgery):The surgical site involve the body of mandible of dog ,it was sterilized by iodine solution (2%),then 3 sided buccle full thickness mucoperiosteal flap was incised and reflected in the area opposite to molar teeth to expose the bone Fig(1),then hole was created apical to roots of present molar by low speed handpiece with round bur with copious irrigation by distilled wated to preper hole (1cm )in diameter Fig(2) ,then repositioning the flap and sutured by 3/0 black silk suture material.

**Part II** , (Autogenous bone marrow excavation): The lateral aspect of femour area was involved in this procedure ,incision of 2cm length in skin ,dissection of muscle and subcutaneous tissue Fig(3) ,the hole was created into femur bone with surgical round bur by straight hand piece into the cortical plate then to marrow space in diameter of (1)cm with surgical excavation of bone marrow Fig (4).

Animals grouping ,dogs were divided into 2 groups as follows,

1- Control group : The number of dogs in this group were (4) ,they received surgical procedure part I only, the hole filled with Hydroxyapatite granules only. Hydroxyapatite granules(500-1000 μm),Genius Baumer S.A. BRAZIL
2- Experimental group: The number of dogs in this group were (4)

,they received the surgical procedure part I and II ,but the mandibular hole filled with mixture of Hydroxy apatite granules and Autogenous bone marrow (excavated from femur bone).

Post operative follow up: Follwing implantation and recovery of animal ,antibiotics is given by IM(Ampiclox Vial 500mg) once dialy till day 7 to control of infection . Sampling of blood (2ml) was taken from femur vein ,one sample preoperatively and then at 1st, 2nd ,3rd and 4th weeks respectively for biochemical analysis of Alkaline phosphatase enzyme (ALP).

## Results

**Group I:** (Hydroxyapatite group):In the 1st week ALP was within normal level which was (18.5 μ/L).In the 2nd week ALP was markedly increased compared to control which was (29.0μ/L).

In the 3rd week ALP although it is increased but still in high level comparing to control (25.0μ/L) .In the 4th week ALP reduced comparing to 3rd week but still higher to that of 1st week(22.1μ/L) .Table (1),Fig (5).

**Group II:** ( hydroxyl apatite with autogenous bone marrow):In the 1st week ALP was within normal level which was (18.3 μ/L).In the 2nd week ALP was markedly elevated compared to control which was (35.8μ/L).

In the 3th week ALP although it is reduced slightly but remain within the highly level comparing to control (28.6μ/L) .In the 4th week ALP reduced comparing to 3rd week but still higher to that of 1st week (25.3μ/L) . Table (1), Fig (5).

Statistical comparison of both groups using t-test to determine the level of significancy , results showed that their were highly level of significancy at day (15) and (21) $p<0.0001$ ,while there were no significant at day (7) and (21) $p>0.005$. Table (2)

## Discussion

Several types of bone graft based on combination of natural and synthetic material have been used successfully,Hydroxyapatite(calcium phosphate)are generally considered material of choice as synthetic bone substitute [16]. Porous hydroxyapatite (HA) manufactured by foaming of aqueous ceramic suspensions and setting .The foams provide tortous frameworks and large interconnected pores that support cell attachment and organisation into three dimensions arrays to form new tissue. The HA foam implant were progressively filled with mature new bone tissue and osteoid after the implanted period,confirming the high osteoconductive potential and high biocompatibility of HA and the suitability of foam network in providing good osteointegration. No immune or inflammatory reactions were detected[17].In the experimental group indicate that bone formation facilitating at the 2nd week of implantation,this demonstrated by markedly increased of serum alkaline phosphatase (ALP), which means that there is increased in osteoblast activity due to the presense of bone marrow cells might be started immmediately after implantation .this means that there is no phase I osteogenesis ,there is only phase II which is started 15 days after implantation and last as the bone remodelling process continous .phase II (in this phase there is intensive angiogenesis and fibrogenesis followed by host bone formation ,fibroblast and mesenchymal bone cells are induced by substance present within the bone that promote

there differentiation of osteoblast which lay down new bone ,these low molecular weight substance called bone morphogenic protein BMP )[18].In this study osteogenesis was faster and rapid elevation of serum ALP in implantation of hydroxyapatite with BMP,with same results obsereved by Kone ***et al*** [11,19] the authors founded that combination of autogenous bone marrow with hydroxyapatite have rapid osteogenesis compare to hydroxyapatite alone ,in that hydroxyapatite with bone marrow have an additional osteoconductive properties mediated by bone marrow stem cells (osteoprogenitor cells)and started to differentiated and begin osteogenesis immedietly after implantation that is why rapidly elevated of ALP in 2nd and 3rd week compare to control.As osteoblastic activity started after 2nd week of implantation as last as the bone remodelling process continous this finding confirmed histologically examination by the researcher indicated that 2nd week which demonstrated active osteoblast in the experimental group (bone marrow)while no osteoblast cells had been seen in control group[11].

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Table (1) Serum alkaline phosphatase in hydroxyapatite group and Hydroxyapatite with Bone marrow group.

| Postoperative days | Hydroxyapatite alone | | | Hydroxy apatite with bone morphogenic protein | | |
|---|---|---|---|---|---|---|
| Day 7 | Mean µ/L | +SD | +SE | Mean µ/L | + SD | +SE |
| | 18.5 | 4.3 | 1.77 | 18.3 | 4.21 | 1.71 |
| Day 15 | 29.0 | 4.3 | 1.60 | 35.8 | 4.30 | 1.59 |
| Day 21 | 25.0 | 3.9 | 1.28 | 28.6 | 3.58 | 1.45 |
| Day 28 | 22.1 | 3.2 | 1.10 | 25.3 | 3.21 | 1.12 |

Table (2) Comparison between control and experimental group

| **Post operative days** | **control group Mean µ/L** | **experimental group Mean µ/L** | **t-value** |
|---|---|---|---|
| Day 7 | 18.5 | 18.3 | 0** |
| Day 15 | 29.5 | 35.8 | -20.3* |
| Day 21 | 25.0 | 28.6 | -40.0* |
| Day 28 | 22.1 | 25.3 | 0** |

*significant p<0.001,**Non significant p>0.05

Fig(1) full thickness buccle flap was reflected in molar area.

Fig(2) Drilling the bone to create hole.

Fig(3)Incision in skin of lateral aspect of thigh .

Fig(4)Drilling the Femur bone to marrow space.

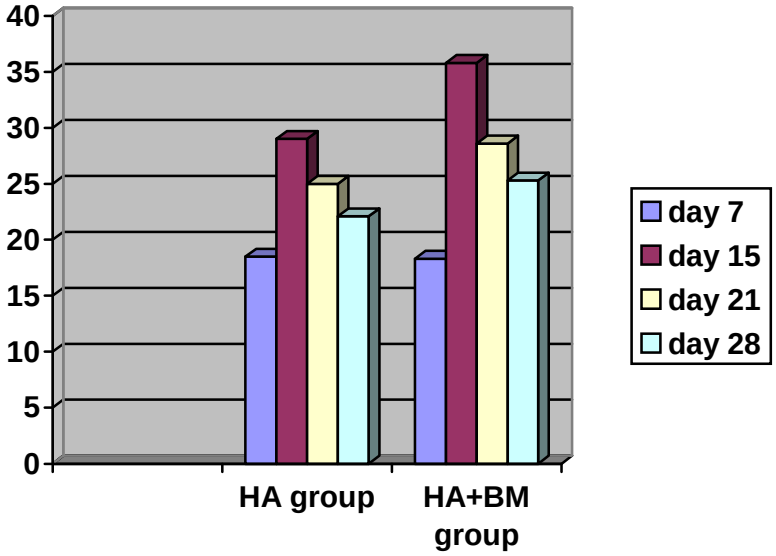


Fig (5) Diagram illustrated direct comparison between experimental and control groups.