The ability of different bone replacing materials to induce bone formation (Experimental Study)

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Abstract:

Many bone replacing materials had been used to manage the problem of large defect following oral surgery, of these materials include hydroxyapatite, bone morphogenic protein, allograft.

In this experimental study a pastes of eggshell powders and calcium sulfate (callus) had been implanted in holes created in the mandible of the six dogs and compared with control holes that filled nothing.

The results were evaluated clinically, radiographically and histopathologically. Clinically at the 7th postoperative day showed that the hole that filled with the egg shell completely healed and difficult to distinguish from the adjacent normal bone. While the hole that filled with calcium sulfate also proved a good healing potential but at less degree from that of eggshell, whereas the control hole was clearly obvious.

Radiographically Osteogenesis was assessed by the degree of opacities in that seems to be clear in the three holes, which made on the mandible (Control, and the treated groups). But seem to be complete opacity in the two treated group, while the control group only seems to be little.

Histopathologically The results showed heavy infiltration of active osteoblasts cells which appear as a sheet of primitive spindle shaped cells surrounding the eggshell materials with immature bone (osteoid) along it .In control hole no osteoblast cell is seen only fibrosis. The hole that filled with callus showed moderate bone activity with little amount of osteoblast cells when compared with egg shell.

Key words:

Bone, Dog, Implantation, Eggshell, Calcium sulfate.

Introduction:

Bone is one of the hardest and strongest substances in the body. Its hardness and strength are due to the association of hydroxyapatite crystals with collagen. Calcium and phosphorus exist in form of hydroxyapatite crystals [Ca_{10} (Po_4) $6(OH)_2$] .It is thought that in the initial phase of mineralization calcium is deposited in the form of a morphous calcium sulfate. It is then transformed to hydroxyapetite by the addition of hydroxy ion[1].

About 90% of the organic component are fibers which are almost type I collagen and Prock1% non collagen proteins.The collagen of bone differ from that of other sites that is because of mineralized and is laid down in bands or lamellae which are roughly paralleled to each other [2].

Bone defects remains as a major concern in reconstructive surgery can be caused by trauma,

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which are the most common one, ablative surgery, congenital distortion and infection [3].

Graft can be defined as living tissue or material that is transplanted and expected to become apart of the host to which it is transplanted [4].

Successful closure of such deformity remains a major role, so that various methods and materials have been advocated, of these using autogenous bone grafts [5], allograft such as decalcified freeze dried bone [6]. More recently considerable attention have been focused on synthetic materials and such materials said to be biocompatible most posses some properties and the most important one is biocompatible to host tissue and able to simulate bone induction [7]. Such synthetic materials posses advantages over the natural autogenous or allogenous graft because the limitation of amount and quality of the last .The limitation have lead to the experimentation and use of variety of materials as a source of bone grafting [8]. The most common synthetic materials used in oral surgery are biopolymer such as poly(amino acid)can be synthesized from natural and chemical derived amino acid[9],porous ceramic of AL_2O_3 [10],calcium sulfate (plaster of Paris) can be used in case with tuberculous osteomylitis [11].Bioactive glass can be bound chemically to bone [12].

The other one is calcium sulfate (Hydroxyapetite) and these materials have received attention in reconstructive surgery in last decades due to highly biocompatible [1].

Bone Substitutes Materials:

There are multiple materials used as bone substitutes for potential clinical use in bone regeneration:

1. Polymer:-

Biopolymers such as poly (aminoacids) can be synthesized from natural and chemical derived aminoacid that may be suitable for bone repair materials. Cow collagen and chemical hydroxy apatite is mixed with patient s marrow into a paste and put into the area of missing bone to encourage new bone growth [9].

2. Alaumina: -

Porous ceramic of Al_2O_3 regarded as highly biomaterial in clinical application, although it is biocompatibility they have not been strong enough mechanically [10]. Examples of that are: -

a) Calcium sulfate. (Plaster of Paris).

Was used to fill bone cavities in patients with tuberculous osteomylitis [11] and in Vietnam War as immediate fill of traumatic bone loss [13].

b) Bioglasses.

Bioactive glass that can be bonded chemically to bone. The main trait of these bioactive glass is the formation of hydroxy carbonate apatite layer on their surface in contact with any aqueous solution. This layer is the equivalent in composition and structure to the mineral phase of bone[12].

C) Hydroxyapatite.

Calcium sulfate materials have received attention in reconstructive surgery during the last decades because they are biocompatible and also capable of forming tight bond with the surrounding bone [14]. Two widely experimental forms are Beta-tricalcium sulfate $[Ca_3(Po_4)_2]$ and hydroxy apatite $[Ca_{10}(Po_4)_1(OH)_2]$ [1].

Materials and methods:

This experimental study was carried out in the college of Veterinary

medicine/Department of Surgery-university of Mosul..

1- Experimental animals:

Six dogs from both sexes, weight 16-24 Kg aged 6 month to 2 year apparently healthy were used in this procedure.

All animals were examined and prepared in one week before the surgical operation. Animal kept in cages in department of Surgery with food and water supply daily.

2- Material and tools:

1. Eggshell: - collected eggshell is fine crushed to make it as powder. And collected in Jar sterilized by autoclave so as to become ready for application.
2. Calcium sulfate(callus) powder: This are prepare from local market and sterilized as in figure (1).
3. Cat gut No.3/0.
4. Surgical tools which are sterilized by autoclave.

Fig (1): Materials implanted in the mandible of the dog.

Surgical procedures:

1- preparation of the animals: -

All animals were fasted before day of operation to prepare them for general anesthesia before operation.

2- Anesthesia: -

Using general anesthetic solutions like: -

ketamine HCl .dose (15mg/ l kg B.W).

Xylzine HCl .dose (5mg/ l kg B.W).Given at same time IM and When animal recover during operation 2nd does was given.

3- surgical operation: -

Mouth opening controlled by mouth gag.the gum is washed with iodine solution, then we made a 3-sided mucoperiostel flap in the lower jaw at the molars region.The flap was reflected using Hawarth periosteal elevator and by using amanual drill 3 holes were made in the mandible of the dog about 0.5cm *0.5cm in diameter and depth as in figure (2).

Fig (2): Preparation of the holes in the mandible of the dog.

The first hole from the midline leaves it with out any material and considered as control while the second hole filled with eggshell powder mixed with distilled water to make it as a paste. Whereas the third hole filled with calcium sulfate powder mixed with distilled water as in figure (3).

Fig (3): Materials implanted in the mandible of the dog.

The surgical site was irrigated, cleaned and the flap was sutured into its position using 3/0 catgut absorbable using simple interrupted sutures technique.

Post Operation follow up:

1-Course of penicillin 10000 I.U/Kg B.W for 3 days was given to each animal subjected to the operation.
2-Soft diet was given to the animal for the first 3 postoperative days.
3-radiographic image was taken at the 5th and, 10th postoperative days.
4-The animal killed after one month, jaw was isolated and site of operation was cut and fixed in formaline 10%.
5-The histopathological procedure was performed to evaluated healing process of jaw.

Results:

1- Clinical study: The animal appears in good health with normal heart rate, Respiratory. rate and normal temp. Appetite of animal seem to be normal, Soft food (milk) was given to animal for 2 days after operation then return to normal food, while free water was given. At the 7th post operative day the flap is opened to assess the healing and the regenerative potency of the implanted materials clinical results showed that the hole that filled with the egg shell completely healed and difficult to distinguish from the adjacent normal bone. While the hole that filled with calcium sulfate also proved a good healing potential but at less degree from that of eggshell.

Whereas the control hole was clearly obvious at the 7th postoperative day as in fgure (4).

These events give a good clinical indication of the compatibility and regenerative ability of the implanted materials in comparison with control hole.

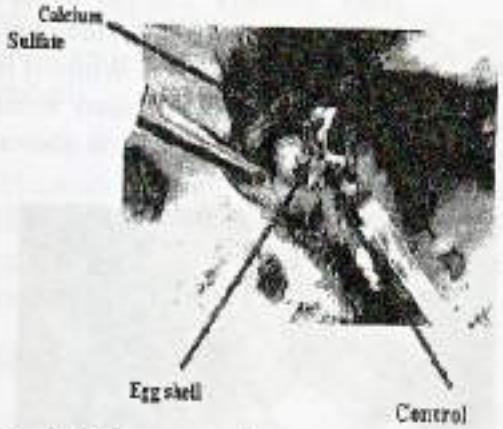


Fig (4): The seventh post operative day following implantation.

2- Radiological study: After 5 days:

Osteogenesis seem to be clear in the three holes which made on the mandible(Control, and the treated groups). But seem to be complete in the two treated group, while the control group only seems to be little these can be distinguish from the degree of opacities. The third holes (eggshell group) seem to be complete filling holes (complete regeneration). It is very difficult to recognize holes the adjacent bone. The 2nd group (calcium sulfate treated group) show good osteogenesis complete filling hole but can easily recognized hole (partial regeneration) as in figure (5).

Fig (5) :Cephalometric radiograph at the 5th postoperative day showed the control hole while it is difficult to recognize the treated holes.

After 10-days Control group: we can recognize hole. However, Claus formation filled hole. While it is impossible to recognize treated holes completely seem to be as bone shown in figure (6).

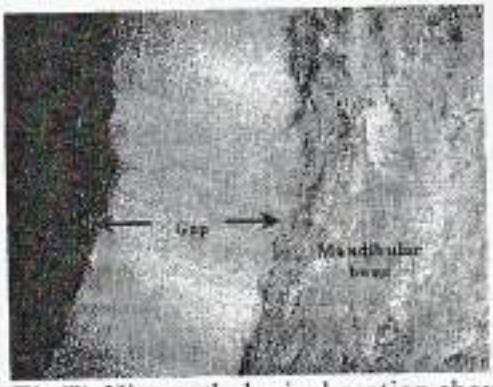
Fig (6) :Cephalometric radiograph at the 10th postoperative day showed the control hole while it is impossible to recognize the treated holes.

3-Histopathologic study:

Biopsies were taken after one month for the three holes. The results showed a lot of active osteoblasts cells which appear as a sheet of primitive spindle shaped cells surrounding the eggshell materials with immature bone (osteoid) along its shown in Figure (7).

Fig (7): Histopathological show good osteogenisis product by eggshell implantation.

In control hole no osteoblast cell are seen only fibrosis as shown in Figure(8).



Fig(8): Histopathological section shows no bon formation in control group.

The hole that filled with callus (plaster of Paris) showed moderate bone activity with little amount of osteoblast cells compared to egg shell material in addition to that fibrosis also seen in figure (9).

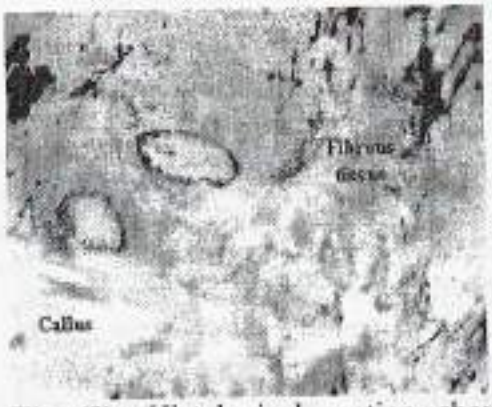


Fig (9): Histological section shows moderate osteogenesis produced by callus implantation.

Discussion:

Many methods and materials have been advocated to use to replace the exsit defect. Autogenous bone graft have been extensively used by surgeons, although it have some disadvantages of donar site such as morbidity, variable resorption and unpredictable remodeling of graft [5]. When there is extensive bone loss and insufficient autogenous bone allograft materials such as decalcified freez dried bone, which is widely available and has osteogenic material but there is slight risk of immunogenicity and pathogenicity [6].

More recently considerable attention has been focused on synthetic materials .Any such material said to be successful most possess some properties in that they must be biocompatible with host tissue, able to simulate bone induction [7]. During the last decade, calcium sulfate materials have received attention in reconstructive surgery because they are biocompatible with capable of forming high bonds with surrounding bone [14].

Our results demonstrated that bone formation take place at the first 4 day of implantation and completed at first 10 days, it means that there is an osteoblastic activity due to presence of bone marrow cells which might be started immediately after implantation. This indicate that phase 1 osteogenesis is started from the survived and the proliferated cells of bone marrow.

We confirm our results by histological examination after one month we observed that highly increased number of osteoblast cells over and around the eggshell and callus implanted materials with immature bone formation.This is in agreement with the [15] and [16], who found that partially minerilized bone (osteoid) formation takes place after foure week of implantation.While in the control group there is less number of osteoblast cells,and little osteoid formation,comparable to that of experimental groups, this is in agreement with that found by [17], who said that the use of osteoinductive materials generate bone formation at higher rate than the normal rate of bone repair. Similar results obtained by [18] they found that calcium sulfate was effective in bone regeneration on both large osseous defects and 'through and through osseous defects. An ideal implant is osteoinductive and osteoconductive causes new bone to form and then supports in to the bony defect.

From clinical and radiological, Macroscopic study we can decided that this two material could be used as implant agent. No serious complication has been found.

References:

1. Gerbino, G, Mamier, G, Berone, S, and Benech: A Hydroxyapatite cement reconstruction in Craniofacial defect. J Oral Maxillofac 1998.
2. Prock, OPD, Jkivirik, ko: The biosynthesis of collagen and its disorder. N Eng J Med 1979; 301: 13-77.
3. Mahesh H, Mankari, Sergi A, Kuzuetor, Bruce Fowlr, Albert Kingman and Gehron: In vivo bone formation by human bone marrow stromal cell effect of carrier particle size and shape 2001.
4. Marx RE, Sunders, TR: Reconstruction and rehabilitation of cancer patient in fonseca R J Davis Welt editors reconstructiove preprosthetic. Oral and Maxillofac surg Philadelphia W B Saunders 1986.
5. Harch HH, Zeithofer HF, Neff A, Suder R: Crainofacial reconstruction with new nor ceramic hydroxy apetite cement J craino-maxillo-facial surgery 1998.
6. Leslie P, Gartner, James L, and Haltt: ColorTextbook of Histology. Saunders company Third edition 1997.

7. Bissada NF, Hangorsky U: Alveolar bone induction alloplasty. Dent Clinic North Am1980; 24: 739-749.
8. JimCrisdale Ba, Dip, Pros,Perio: The clinical application of synthetic bone alloplasts. J can dent Asso 1999; 65: 559-562.
9. Stupp SL, Cicyler, Gw: Organo apatites materials for artificial bone synthesis and microstructure J Biomed Res 1993; 26: 169-178.
10. Klawitter JJ, Hulbert SF: Application of porous ceramics for the attachement of losd bearing internal orthopedic application. J Biomed mater res 1971; 2(1):161.
11. Peltier LF: The use of plaster of Paris to fill defects in bone clin orthop 1961; 21: 1-31.
12. Revaglioni A, Krajewski A: Bioceramic London chapman Hall press 1992.
13. Kelly JK: Maxillofacial missile wounds evaluation of long term results or rehabilitation and reconstruction. J Oral Surg 1973; 31: 438-445.
14. Denissen HW: Dental root implants of apatite ceramic PhD Disseration. Free university Amsterdam the Netherlands 1979.
15. Kenneth R, John D, Zardiacks R, Robert D, Teasdall G, and Scott J: Evaluation of the efficacy of synthetic bone graft substitute in weight bearing canine femoral defect model. Zimmer Medical Research Collagraft Zimmer inc WarsanIndiana 1997.
16. Okumura M, Ohqushi H, Dohi Y, and Katuda T: Osteoblastic phenotype expression on the surface of the hydroxyapatite ceramics J Biomed Mater Res 1997; 37(1): 122-129.
17. Kone E, Muraglia A,Corsi A, Bianco P, and Boyde A: Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical –size defects of sheep long bones J Biomed Mater Res Mar 2000; 5:449(3): 328-337.
18. Murashima Y, Yoshikawa G, Wadachi R, Sawada N, Suda H: Calcium sulfate as a bone substitute for various osseous defects in conjunction with apicectomy. Int Endod J Sep 2002; 35(9): 768-774.