

Histopathological evaluation of aragonitic calcium carbonate as a bone graft substitute in rabbit's mandible (An experimental study)

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ABSTRACT

Background: Augmentation of the craniomaxillofacial region is required for many aesthetic and reconstructive procedures. Many bone graft substitutes have been developed. These products differ in their osteoconductive and osteoinductive properties. The use of bone graft substitutes offers the ability to lessen the possible morbidity of the harvest site in autograft. Aragonitic calcium carbonate has proved to have biocompatible properties as bone graft substitute. This study aimed to evaluate the histopathological effects of aragonitic calcium carbonate in experimentally induced bone defect in rabbit's mandible.

Material & method: Twenty five adult male healthy rabbits were used in current study. Two holes were prepared in the rabbit's mandible, first hole remain empty as control, the second filled with aragonitic calcium carbonate. Samples were taken after 2, 6, 8, 12 and 24 weeks. Histopathological evaluation include; examination of new bone formation and inflammatory response, in both control and experimental groups.

Results: At 2 weeks, there was no new bone formation in both defects with moderate amount of inflammation. At 6, 12 and 24 weeks the amount of new bone formation in implant group was more than that formed in control group (statistically highly significant correlation, $p < 0.01$). At 8 weeks, less bone amount in implant group. There were marked degradation of implant particles at 6 weeks.

Conclusions: ACC is suitable bone graft substitute and induce new bone formation by acting as bioactive, osteoconductive bone graft when come in contact with bone.

Key words: Natural coral, bone graft, calcium carbonate. (J Bagh Coll Dentistry 2012; 24(4):58-61).

INTRODUCTION

Bone tissue in the human body comprises the largest proportion of the body's connective tissue mass. This tissue unlike other connective tissues, its matrix consists of physiologically mineralized, tiny crystallites of a basic, carbonate-containing calcium phosphate called hydroxyapatite distributed in an organized collagen structure. ⁽¹⁾

Large traumatic bone defect can be covered by soft tissues but reconstruction of the bone itself may be difficult. The use of autograft material remains the gold standard for use in orthopedic settings due to its osteoinductive, osteoconductive, and osteogenic potential. ⁽²⁾ Augmentation of various congenital and acquired defects of bone tissue is a topical problem in oral and maxillofacial surgery. Around half of the patients requiring dental implants has severe alveolar bone deficit which can be replaced using bone grafting from the patient, biomaterials or combination of both. Biomaterials of different origins as natural and synthetic ones have different mechanisms of host response. Biomaterials should be (1) stable, (2) biocompatible (3) ideally osseoinductive (4) osseoconductive (5) porous (6) similar to biological bone mechanically. ⁽²⁾

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Porous calcium ceramics have proved to be biocompatible bone substitute. The original coral skeleton, consisting of calcium carbonate, can also serve as bone substitute. The main difference between these structurally identical materials is that biodegradation takes place much more slowly with HA than with calcium carbonate. ⁽³⁾

MATERIALS AND METHOD

Twenty five local breed adult male healthy rabbits of average weight (1, 5-2) KG were used kept under standard condition. The animals were under supervision of the veterinarian staff at the veterinarian hospital in Nassiriyah. Six capsules of aragonitic calcium carbonate (Novocor Plus (BO) CO. Italy) were used in current study. The product is contained in capsules with a dose of 500 mg each. Using the doser provided, add two drops of blood of rabbit to product. The contents inserted between the forks of an amalgam vibrator and vibrated for minimum time of 1.5 min. or 2 min. The rabbits were divided into five groups according to the healing period (2, 6, 8, 12 and 24 weeks). Five rabbits were sacrificed for each of the five periods. Animals received Ivermectin injection to prevent external and some internal parasite infection 2 days before surgery and the dose repeated after 2 days, one hour before operation each rabbit had

systemic administration of antibiotics. The surgical procedures were done under general anesthetic drugs. By using atropine sulfate as premedication to reduce salivary and mucous secretion, followed 10 minutes later by mixture of ketamin hydrochloride 10% and xylazin 2%. I.M Lidocaine hydrochloride 2% was infiltrated subcutaneously along the planned surgical site (extraorally) ⁽⁴⁾.

All instruments were sterilized in an autoclave kept at pressure of 15 lb/sq in above atmospheric pressure to obtain a temperature of 121 C for 15 minutes ⁽⁵⁾.

An incision was made along the external aspect of body of mandible at right side in the naturally edentulous space between the incisors & premolar teeth. The size of the cavity approximately 5mm in diameter and 2mm in depth ⁽⁶⁾, two holes were prepared, The first hole (anterior) remain empty as control, the second hole filled with aragonitic calcium carbonate. Immediately after resection of bone, the specimens were divided into two segments (one control and one experimental), then fixed in 10% buffered formalin for 48 hours. After fixation decalcification with solution of 10% formic acid was made. After decalcification, the specimen was dehydrated by graded series of alcohols 70%, 80%, 90% and absolute alcohol. Then clearing by xylene. The processed tissue was impregnated in paraffin wax. The paraffin embedded sections were cut in rotary microtome and mounted on glass slide, dewaxing and immersed in xyelen then stained with H&E as routine slide stain.

Histopathological examination include; evaluation of bone apposition, neovascularity, type of material reaction with bone and degree of inflammation were assessed.

On an ordinal scale, sections were scored for bone ingrowth in control & experimental groups in two healing parameters:

- (i) New bone amount according to ⁽⁷⁾ grading system table(1)
- (ii) Inflammation according to ⁽⁸⁾ grading system table(2).

All data of the sample were subjected to computerized statistical analysis using SPSS version 15 (2006) computer program. The statistical analysis included:

1-Spearman rho; to compare the difference in inflammation and bone formation in each interval.

2-Pearson chi-square; for comparison among groups.

Table 1: Grading of bone healing (new bone) for Histological evaluation. ⁽⁷⁾

New Bone: amount	scoring
None	Score 0
Scattered islands	Score 1
Thin sheet	Score 2
Bone trabeculae	Score 3

Table 2: Grading of bone healing (inflammation) for Histological evaluation ⁽⁸⁾

Inflammation	Inflammation	scoring
Severe	Abundant macrophage, giant cells and PMN leucocytes	Score 3
Moderate	Many macrophages/giant cells with few PMN leucocytes	Score 2
Some	Few macrophages/giant cells	Score 1
None	No inflammation	Score 0

RESULTS

Experimental group (two weeks):

Histopathological findings of experimental group at two weeks showed the particles is dissolved by decalcification process. The defect appears empty spaces except of that. There were evidence of moderate inflammation with connective tissue formation. Osteoblastic activity at the bone periphery. There was no new bone formation (Figure 1)

Control group: Evidence of moderate degree of inflammatory response. No new bone formation

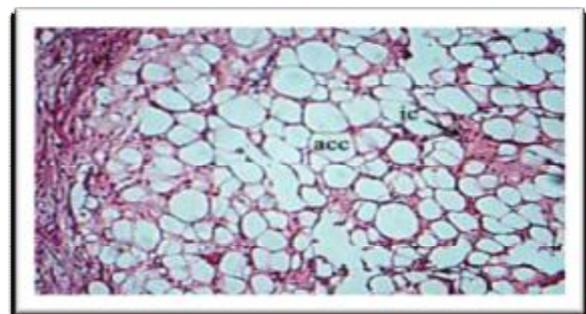


Figure 1: Histopathological section of experimental group at 2 weeks showed empty spaces of decalcified implant material (acc) with inflammatory cells (ic). (H&E20X)

Experimental group(six weeks):

Histopathological findings of experimental defects at six weeks showed osteoblastic activities producing thin sheets of immature bone at the periphery and inside the defect and particles. Thin fibrous connective tissues were formed, there. Few empty spaces represent decalcified materials (Figure 2)

Control group: formation of scattered islands of bone with mild degree of inflammatory response

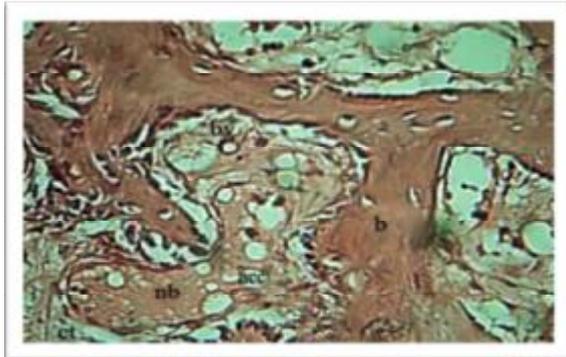


Figure 2: Histopathological findings of experimental group at six weeks interval, showed osteoblastic activity (ob) at periphery of bone (b) with new bone formation (nb). Implant appear as empty spaces (acc) . Amount of collagen fibers (ct) & blood vessel (bv)(H&E 20X)

Experimental group(eight weeks):

Histopathological observations showed marked deformity of the implanted material. With gradually increasing bone formation of irregular, thickened and haphazardly bone ingrowth that replace the matrix of resorped implanted material core.(Figure 3)

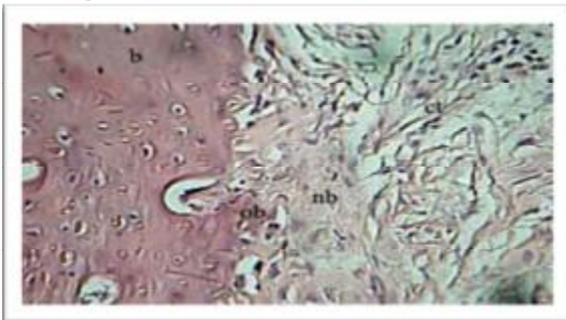


Figure 3: Histopathological findings of experimental group at eight weeks interval, showed osteoblastic activity (ob) at periphery of bone (b). With formation of new bone (nb), connective tissue (ct). Section showed marked resorption of implant.(H&E20x)

Control group: Formation of new bone; consist of few amount of scattered islands and large of thin sheets.

Experimental group(twelve weeks)

Histopathological evaluation revealed complete resorption of particle, with large new bone formation as well as interparticular matrix (Figure 4) .

Control group: the defect filled with thin sheets of mixed woven and trabecular bone

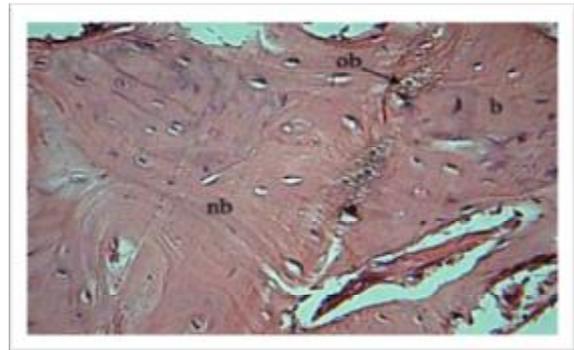


Figure 4: Histopathological findings of experimental group at twelve weeks interval, showed osteoblastic activity (ob). With formation of new lamellar bone (nb) . With complete resorption of implant. (H&E20x)

Experimental group Twenty Four Weeks:

Histopathological observations revealed complete healing with new bone formation. The defect filled with mature bone, Osteocyte located with lacunae. Complete biodegradation of particles during this interval was seen and completely replacement by new bone ingrowth.

Control group: There was large amount of lamellar bone formation filled the defect with.

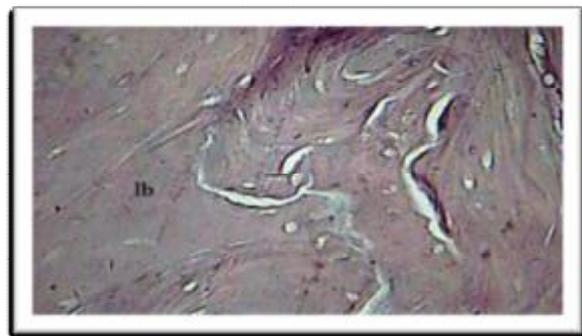


Figure 5: Histopathological findings of experimental group at twenty four weeks interval, showed new lamellar bone formation (lb) with complete resorption of implant.(H&E 20x)

Correlation of new bone formation and degree of inflammation in control and experimental groups regarding time intervals.

Results of present study revealed statistically highly significant correlation ($0.01 \geq P > 0.01$) between control and experimental groups concerning amount of new bone formation in the intervals of 6, 12, 24 weeks, while statistically non significant correlation in interval 8 weeks (figure 7)

Regarding degree of inflammation ,results showed statistically non significant correlation among control and experimental groups in all periods(figure 6)

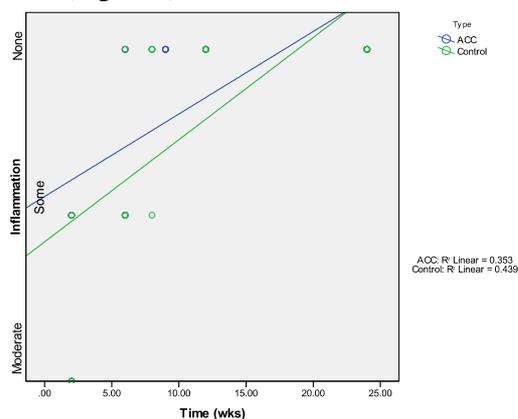


Figure 6: Diagram show not significant statistical relation of inflammation among study groups .Green line represent control group. Blue line represent implant group

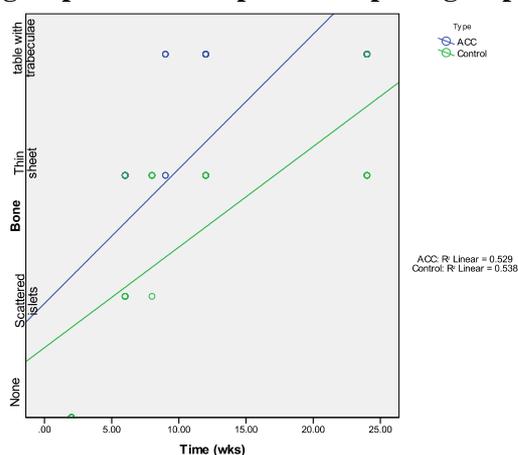


Figure 7: Diagram demonstrate statistical relation of new bone formation among study groups. Green line represent control group. Blue line represents implant groups

DISCUSSION

At the end of the second weeks, both groups showed moderate degree of inflammatory response .No significant statistical differences ($p=0.527$) between the experimental and control groups at this interval, this due to, each animal

acts as its own control, allowing the examination of individual response characteristics.

At six weeks, there was evidence of resorption of implant particles because Calcium Carbonate loses its internal porous structure very quickly and after that the bone does not actually invade the pores but replaces the matrix. Histopathological findings at eight week showed that bony ingrowth occurs at the bone interface and osteoconduction of new bone along the implant matrix. At 12 weeks the study showed, the degradation of particles are complete. At 24 weeks the bone defect in the experimental group filled with mature (lamellar bone) with complete biodegradation of biomaterial particles, these data illustrate the osteoconductive properties of ACC particles as well as high rate of degradability and clearance from the sites.

The fact that the amount of bone formation with a direct bone interface with surface of implant particles are indicated to us that the coral particle, will be suitable biological based bone graft substitute for oral and maxillofacial reconstruction in the human beings and in the clinical application services.

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