Clinical and experimental Study to evaluate the effect of Biphasic calcium phosphate collagen composite (cpcc) on healing of bone defects after oral surgical procedures

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ABSTRACT

Background: A recent discoveries used for reconstruction in maxillofacial surgery is the composite bone graft materials. The availability of collagen I matrix make our choice to use this material in surgery .It is biomaterials that its structure could be modified by simple techniques. Studies to find a new materials use for bone reconstruction is to overcome the disadvantages of autogenous bone and the synthetic resorbable bone substitutes.

Objectives: This study was done to evaluate the effect of biphasic calcium phosphate collagen composite (ccpc) on healing of bone defects after oral surgical procedures.

Type of the study: A cross sectional study.

Method: It involved 60 patients, 35 male and 25 female, age (15-40) yearsand experimental animals study involved sixty rabbits.The study was done in Department of Maxillofacial surgery in Al anbarCollege of Dentistry (2015-2016).These patients were received tooth extraction, and other minor surgical procedures .Clinical and radiographical examination and patients consents are done before surgery. The surgery was done under local anesthesia.The resulting bone cavities are filled with composite bone graft material composed from combination of lyophilized hydrolysed collagen sponge (Hemospon),Brasil and Osteon II bone graft material,Korea .The area is closed with 3/0 block silk suture .Antibiotic cover (Ampicillin 500mg ×4) for 1 week was prescribed. The patients were followed up by careful clinical examination with radiograph 15-30 days after surgery. .Sixty New Zeleandi White rabbit were used in our study for histopathologicalexamination.Intraperitoneal injection of ketamine 10 mg with XYL-M2 solution 20 mg xylazine base used to anesthetize the animals. (5mm diameter) bone defects were made using straight surgical hand piece and bur in anterior mandibular region and filled with each material. The study design involve the following groups: group 1(involve 15 rabbit; the defect was made and filled with blood clot only (control group),group 2(involve 15 rabbit, the defect was made and filled with collagen sponge only),group 3(Involve 15 rabbit, the defect was made and filled with Osteon II bone graft only),group4(involve 15 rabbit, the defect was made and filled with Osteon II bone graft +collagen (Composite bone graft).The flap were closed 3/0 black silk suture. The rabbits were killed after 30 days. 10 cmexcisional biopsy specimen was taken from the paramandibular area and placed in 10%formalin then send histopathologicalexamination.

Results: Clinically, the treated area show good healing with absence of infection .Radiographs during follow up period show radiopacity indicating new bone formation .The results of experimental animals (Rabbits) indicategrowth new bone . In group 1 and group2, the center of the defects was depressed by surrounding tissues Eung et al, While, in group 3&group 4, the graft materials maintained the space and the center of the defect did not depress. Statistical analysis indicates that during 4 th week ,the highest healing % is (100%) in group 3 &group4 in compared to control group (50%)..A significant difference in healing % was found among the treated groups.

Conclusion: The result indicates that biphasic calcium phosphate collagen composite can be efficiently utilized clinically.

Key words: Biphasic Calcium Phosphate Collagen Composite, Hydroxyapetite,BetaTricalcium Phosphate, Biphasic Calcium Phosphate.

Al-Kindy College Medical Journal 2017: Vol.13 No.2
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Received 17th April 2016, accepted in final 12th January 2017

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A common transplanted tissuein the body is bone after blood. About 2 million grafting surgeries are performed per year over world to for bone reconstruct in orthopaedics,neurosurgery,and maxillofacial surgery[1].Treat bone defects now by natural or synthetic substitutesallowing tissue growth. Severalbioscaffoldsare usedrecently, the search continues in future in an attempt to overcome the side effectsof the present ones. Type I collagen with its structure could be modified by relative simple techniquesis a fast and cheap way to invent new ones [2]. Multiplicity of grafting surgeries and problems of autograft and allograft treatments (e.g. Limited quantity, transmission of diseases) make the need foralternatives to treat bone defects. Synthetic biomaterials are of importance to be use in surgeryto replaceautogenousbone. These current biomaterial is evaluated based on their ostogenic,osteoinductive,orosteoconductivity[3][4].Osteoi nduction is a bio mechanism that already occurs during

healing. It is starts directly after the bone injury. This process induces primitive, undifferentiated and pluripotent cells to develop the bone forming cells [5].Various materials available today to reconstruct bone defects in surgical field. The first choice one is the autogenous bone, however, has disadvantages e.g., donor site morbidity and uncontrolled resorption[6]. Attempts are made to develop new bone graft materials to replace the autogenousbone.Among these materials e.g. Calcium phosphate (Ca-P) bone grafte.g.,HA and beta tricalcium phosphate (β-TCP) used due to their structural characteristics similar to human bone. It good biocompatible and osteoconductive bone graft material. Among these materials, HA,which is very stable,and very effective e.g. Maintain the space ,but it is low osteoconductive[7][8].β-TCP, is unlike to others, is more biodegradable and quickly replaced by new bone but has low space maintaining capacity. So that biphasic calcium phosphate (BCP),(i.e. and β-TCP), use with success to overcome drawback of other materials[9].BCPs (Particle type) have been frequently and efficiently used but has limitations.These materials are susceptible to pressure by external forces if not protected properly and collapsed easily in non-contained defects. So that, the use of particulated BCPs developed to provide stability in such situations. Despite of that block type BCPs should be adapted ,trimmed to desired shape to, trimmingand secure properly that very difficult in surgery .Some studies re-ported limited bone related to block type BCP.BCP collagen composite (BCPC)graft material introduce today as bone substitutes[10].Collagen sponge is made from animal skin, collagen whipped and baked into sponge. It fills cavities during surgery to stop bleeding [11].Hemospon is lyophilized hydrolyzed collagen. It is porous gelatin sponge. It is fully resorbed within 15 day[12]. It has good hemostatic and healing ability to use in surgery. It also used in oral surgery for augmenting the palatal connective tissue donor sites alone or with platelet concentrate in multiple root-coverage procedures [13]. Because collagen is a major organic constituents of the bone, has several properties i.e., includinghemostasis,chemostactic activity to attract fibroblast, and promotion of wound stabilization which favor healing of bone. Despite of that collagen has limitation such as rapid resorption by the activity of leukocytes,macrophages,andbacteria,resulting in failto space maintain for new bone in growth. The addition of collagen to bone substitute may overcome its weakness [14]. BCPC be easily trimmed into the desired shape due to flexibility of the collagen .Collagen can also favor healing process by maintain blood clots and induce fibroblasts migration.BCPC for that reason, expected to have good bio mechanical property to form bone and easily to be handle clinically, unlike other materials[15].

Materials and Method:Sample: A cross sectional study involved 60patients, 35 male and 25 female, age (15-40) years. The study was done in Department of Maxillofacial surgery in Al Anbar College Of Dentistry (2015-2016).These patients were come for tooth extraction,apiocectomy,and surgical removal of impacted teeth and other surgical procedures.The patient were kept under thorough clinical and radiographical examination before surgery.

Materials: Hemostatic sponge made of Lyophilized HydrolysedCollagen (Gelatine),Brasil,Osteon (II) Lifting synthetic highly resorbable bone graft,korea was used in our study.

Method: Patients consents was taken before surgery.Thepatients were anesthetized by (2% Xylocainewith 1:80.000 adrenaline) local anesthesia. The surgery weredone and the resulting bone defects(Fig.1)arecleaned with normal saline and filled with composite bone graft composed (Fig.2-3)from combination of lyophilized hydrolyzed collagen sponge (Hemospon) and Osteon II bone graft (composed from Hydroxyapatite HP+ Beta tricalcium phosphate β- TCP). The area is closed with 3/0 black silk suture (Fig. 4). Antibioticscover (Ampicillin 500mg×4)1 week was prescribed. The patients were followed up by careful clinical examination with radiograph 15-30 days after surgery(Fig.5).Sixty New Zealand rabbits used forhistopathological examination.Intraperitoneal injection of ketamine 10mg with XYL-M2 solution 20 mg xylazine base is used for anesthesia. Three sided flap were made in the anterior mandible of the rabbits. The bone defects (5 mm diameter) were made using straight surgical hand piece and bur with constant irrigation by normal saline and filled with each materialand allow healing 2 weeks. The study design involves the following groups:

1-group1: involve 15 rabbit: The defect was made and filled with blood clot only (Control group).

2-group 2: involve 15 rabbit, the defect was made and filled with collagen sponge only.

3-group 3: Involve 15 rabbit, the defect was made and filled with Osteon II bone graft only.

4-group 3: Involve 15 rabbit, the defect was made and filled with Osteon II Bone graft +Collagen (composite bone graft). The flap were closed 3/0 black silk suture. The rabbits were killed after 30 days .10 cmexcisional biopsy specimen was taken from the paramandibular area and placed in 10%formalin then sent for histopathologicalexamination(Fig.6).

Results:

1-Clinical examination:

During follow up period the treated area show uneventful healing. With absence of infection and complication such as exposure of graft material (Fig.1-4) (Table1-4).

2-Radiographical examination:

The treated group during the follow up show radiopaque area indicating osteoidformation (Fig.5).

3-Histopathological examination:

Biopsy from treated animals shows osteoid formation (Fig 8-12). The amount of new bone isexcellent in group 4 in comparison to other groups(Fig.11,12)(Table.1). In the group1 and group 2(Fig.7-8), the defect is depressed by surrounding tissues. In comparison with group 3 and group4 group(Fig.9-12), the defect is filled by the graft material (space maintenance) did not depressed .And

the augmented area was higher significantly in group 3 and group 4 in comparison to group 1 and group2(Table.1-2) .

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4-Statistical analysis: The statistical analysis was done by the use of SPSS program.

Discussion: Biocomaptible ,osteoconductive ,osteoinductive potential of bone substitutes should be similar to human bone. These materials provides scaffold for blood clot stabilization, maturation, andmaintain space for growth of new bone, from a clinical point of view, it should be easily handled and suitable cost [(16).]In the present study , the effect of BCPC were evaluated in healing of bone using rabbit mandible bone defect(5mm).Recently the combination of collagen with other substitutes had importance in bone regenerative therapy in oral surgery[(17).]Table (1) show that during 1st week the healing % was the same among all the treated groups 5%, while during the 2nd and 3rd week the healing % is increased 15% in collagen group (group2) and 30% in Osteon II (group3) and graft collagen (group4) .In the 4th weeks the healing percentage was 70% in collagen treated group, while it is the highest 100%in Group3 and group4 when compared with control. Table (2) show significant difference in healing % among the groups.This results is consistent to the results of other study which indicate that, a major advantageous properties of collagen in surgery, in addition to be a major organic composition of bone i.e., it promote hemostasis, chemotactic activity to attract fibroblasts, and promotion of wound stabilization[(18).]In addition, collagen has proven to regulate genes responsible for expression and proliferation of bone forming cells .One of disadvantages of collagen is fast resorption by action of macrophages, leukocytes and bacteria causing failure to maintain space for growth of new bone.Although,the combination of collagen with synthetic resorbablesubstitutes compensate its weakness[(19).]BCPs is a particle type bone graft it used in surgery, due to good bone formationability and maintain space for bone ingrowth due to three dimensional microstructure. Thecombinations of collagen to particle type BCP it provide volume stability and provide scaffold promote fibroblast proliferation [(20).] Many studies used rabbit calvarildefects (10-15mm) i.e. criticalsize, to evaluate the potency of syntheticgraft due to favorable quality and amount of bonemarrow. A circular bone defect size 8 mm is more useful to assess healing and regeneration of bone. But in studies using 8 weeks healing period show limited bone healing [(21).]In this study .5mm circular defects were made in the rabbit mandible and bone healing was evaluated at 2 to 4 weeks after surgery,respectively.Histopathological examination of group 2 show that collagen sponge was totally resorbed within 2 week healing period[(22)(23).]In group3 and group 4 the biopsy resultsindicate that ,graft material show slow resorption and effective in maintain the space, which is consistent with other previous studies. This result indicate that BCP and BCPC show favorable space maintaining capacity for bone ingrowth which is important to maintain blood clot and prevent gross epithelial growth . During the follow up, the amount of bone formed increased. At 1st, 2nd weeks healing periods, there was no significant difference among all groups (Table.1)[(24).]In spite of,BCP graft materials are commonly used bone graft, its capacity for new bone formation increases when BCPs used in various defect models[(25).] Recent trend to improve bone forming capacity of BCP graft materials using BMPs (Bone Morphogenic Proteins).High magnification observation for treated area, the new bone was found in close contact with BCP particles in group3 and group4.This provides evidence that BCP and BCPC materials have good compatibility and osteoconductivity.Our study demonstrate that bone healing and regeneration pattern are similar in group3 and group 4(Fig.13)(Table.1)Both also are similar in total amount of bone formed and augmented area. This indicates that both materials are excellent bone substitutes [(26)(27).]Studies continues to prove that BCPC are superior over BCP in the treatment of non-contained defects. Collagen is a component of BCPC,is used as a carrier of bioactive materials. Recent findings that BCPC can be immersed with growth factors. This provides evidence that BCPC could be used as excellent carrier for large bone defects or non-contained where space maintenance becomes important [(28).]

Conclusion:BCPC and BCP material have good space maintaining capacity and osteoconductivity,indicating that BCPC can be used in various clinical applications effectively. Clinically, thestudy groups show good healing with absence of infection and graft exposure. Radiographs showradiopaque area indicating new bone formation.Theresults of biopsy specimen show new bone formation in the entire treated group with group 4 show the highest space filling capacity compared with other groups.

Acknowledegment:I give special thanks to supervisors in department of maxillofacial surgery of Anbar university and supervisors in department of pathology in Al-Kidney college of medicine and veterinary medicine college of Baghdad for their help and encouragement .

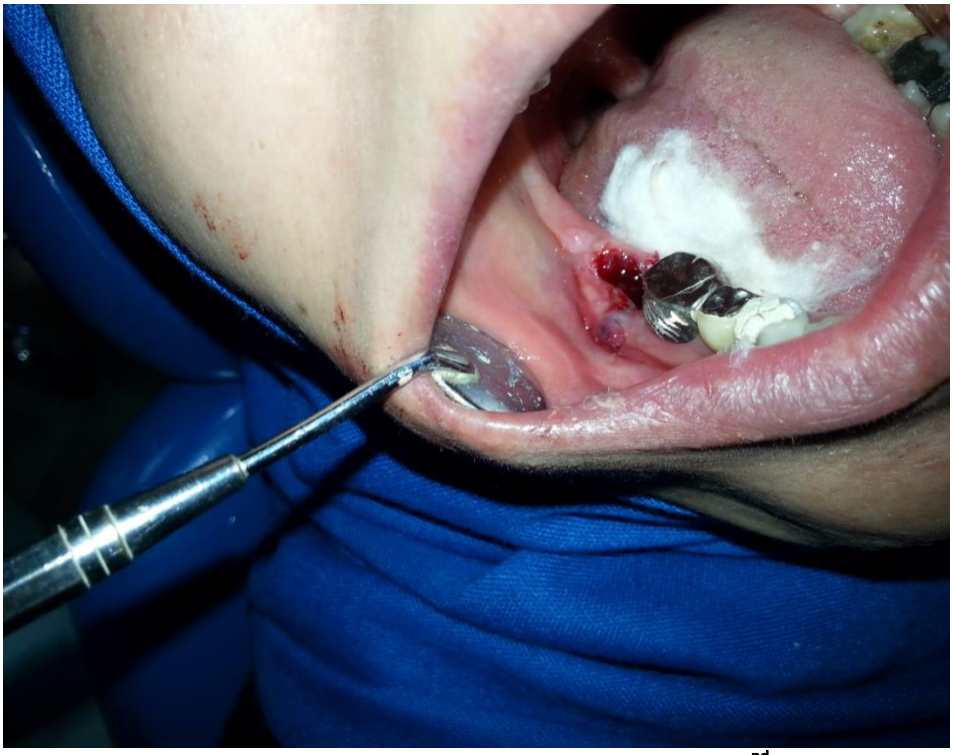

Figure (1): The tooth socket following the extraction of lower 2nd molar tooth.

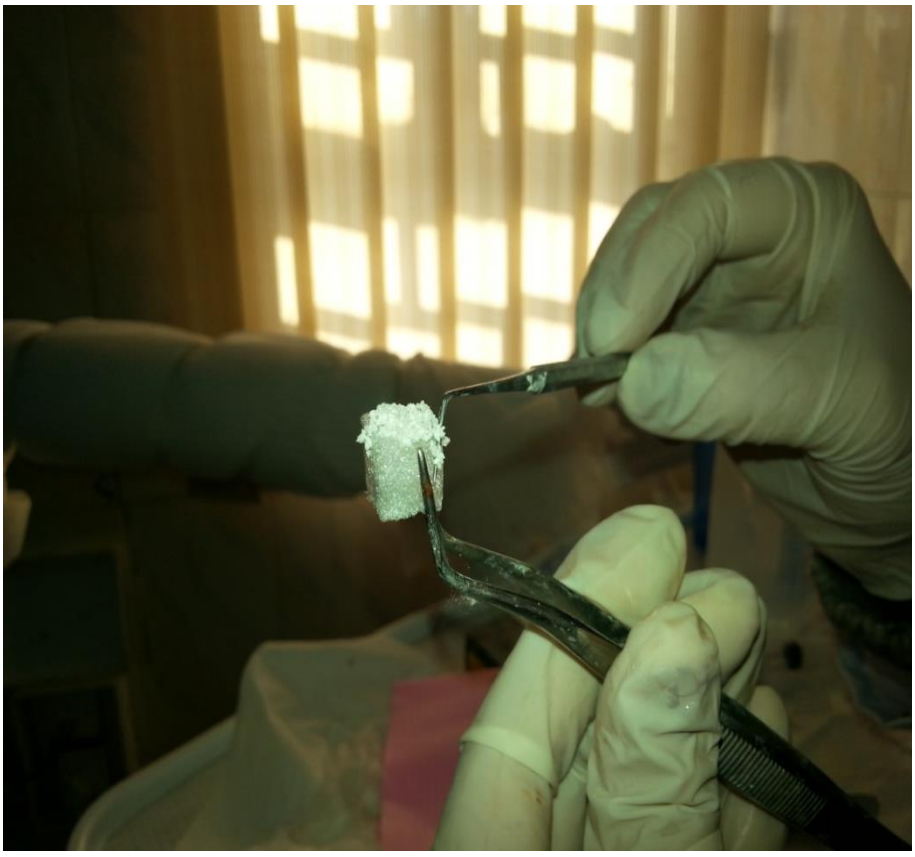

Figure (2): The collagen sponge with the bone graft.

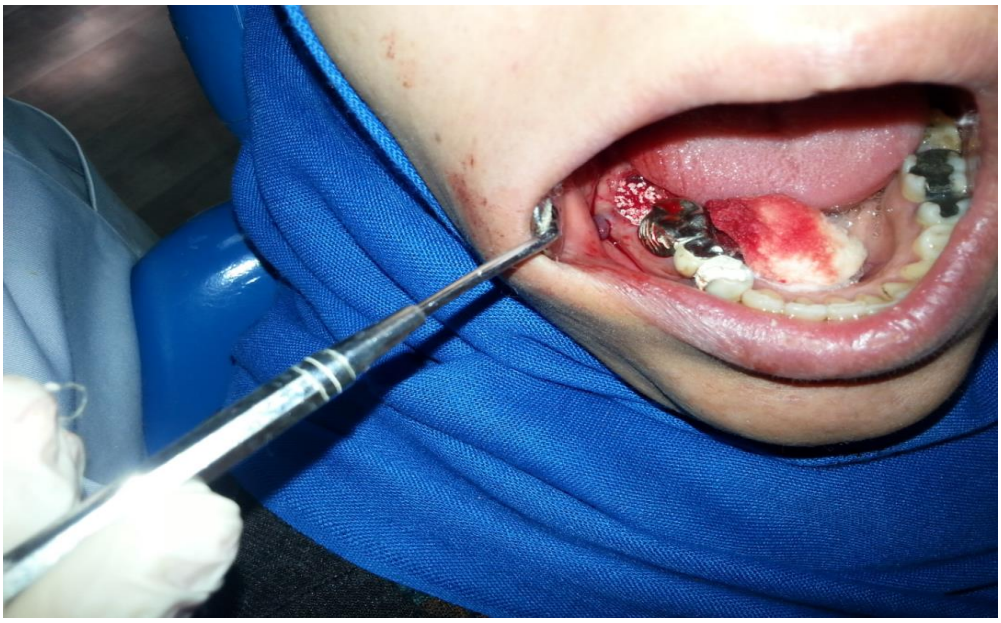

Figure (3): Show the composite bone graft material.

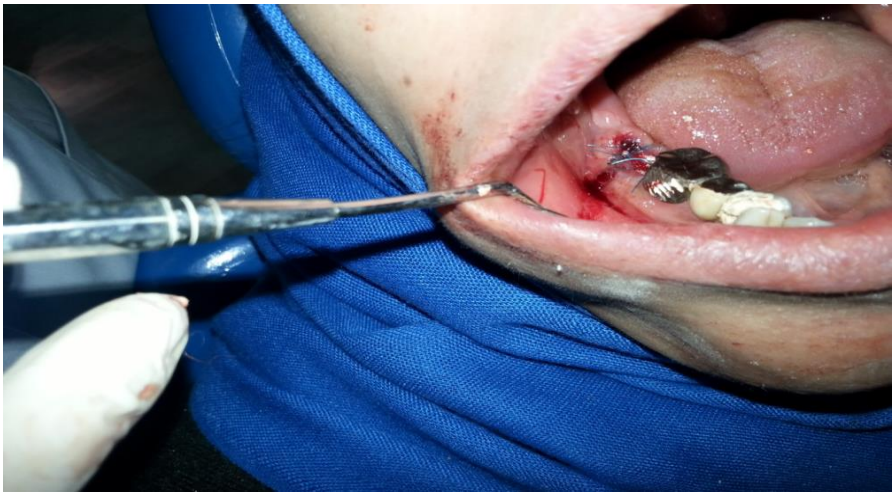

Figure (4): The area after complete treatment and Suturing with resorbable suture.

Figure (5): The postoperative X -ray after 1 week.

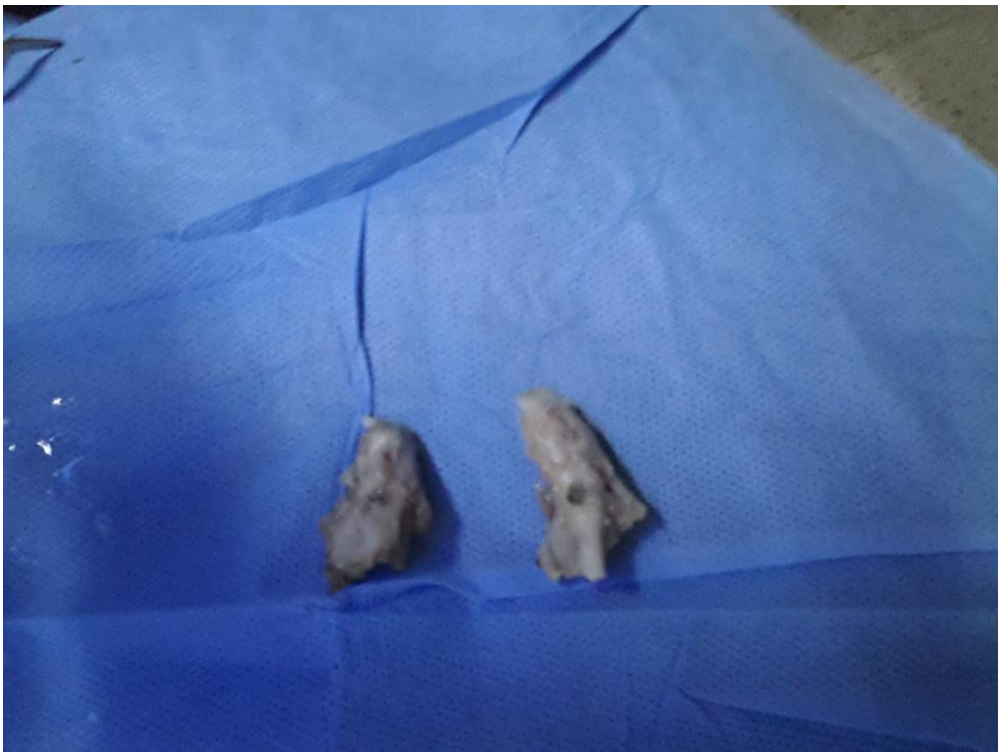

Figure (6): Show the specimen

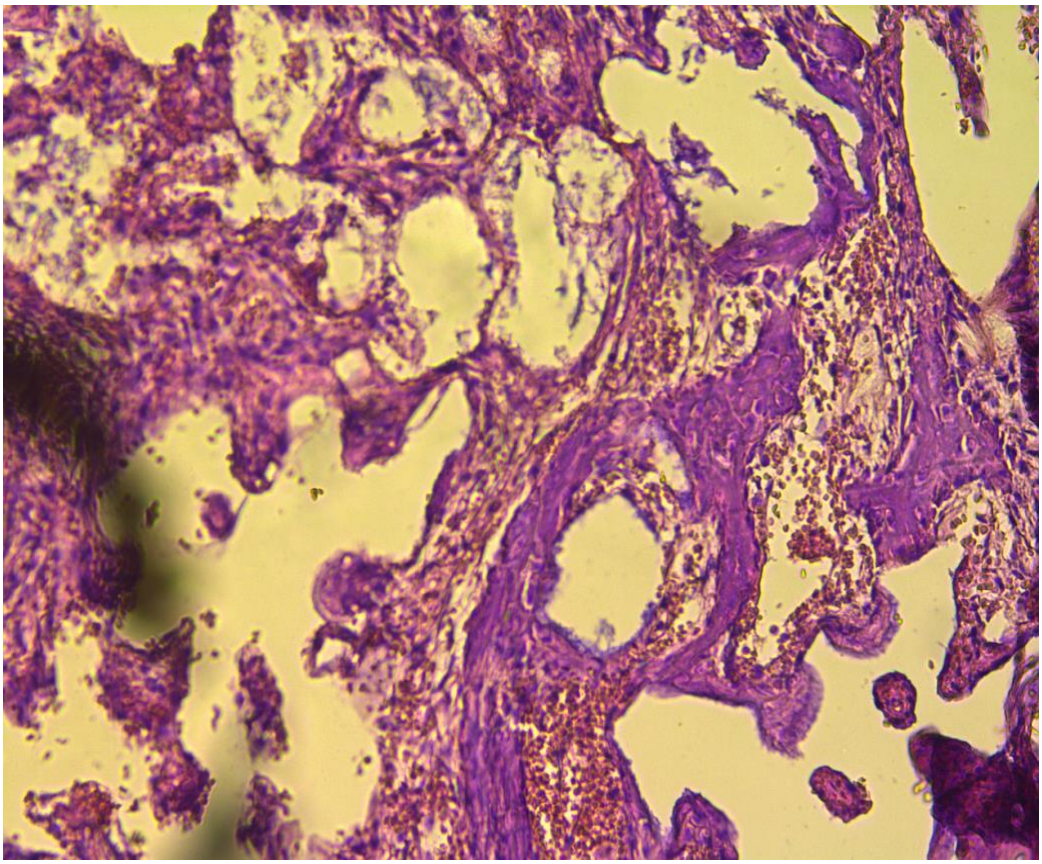

Figure (7):The blood clot in group 1 after 2 weeks

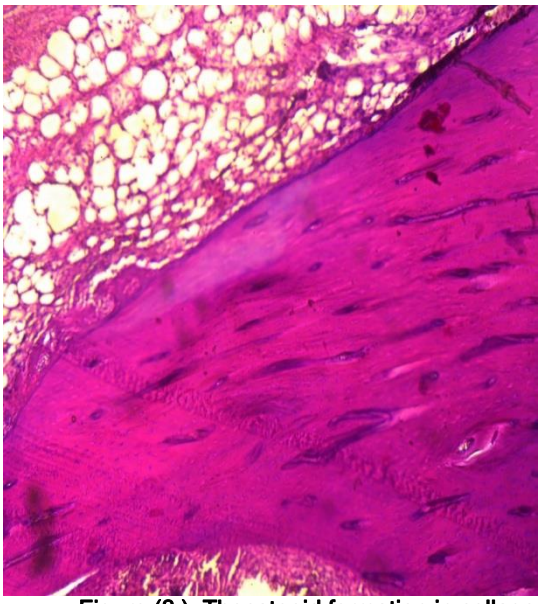

Figure (8): Theosteoid formation in collagen treated group after 2 weeks

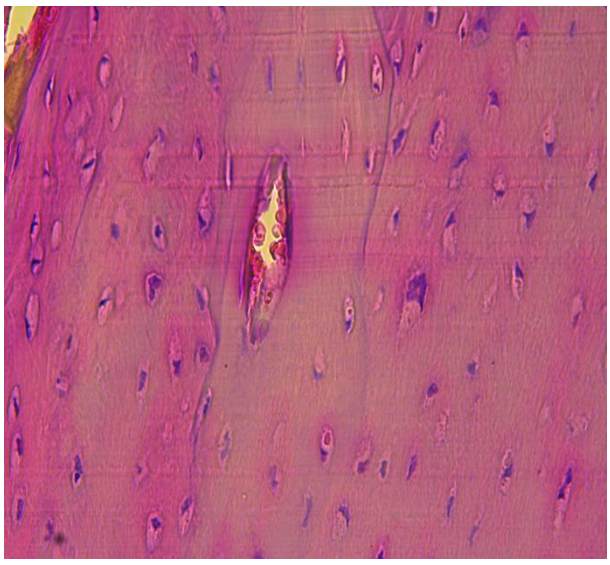

Figure (10): The osteocytes in group3.

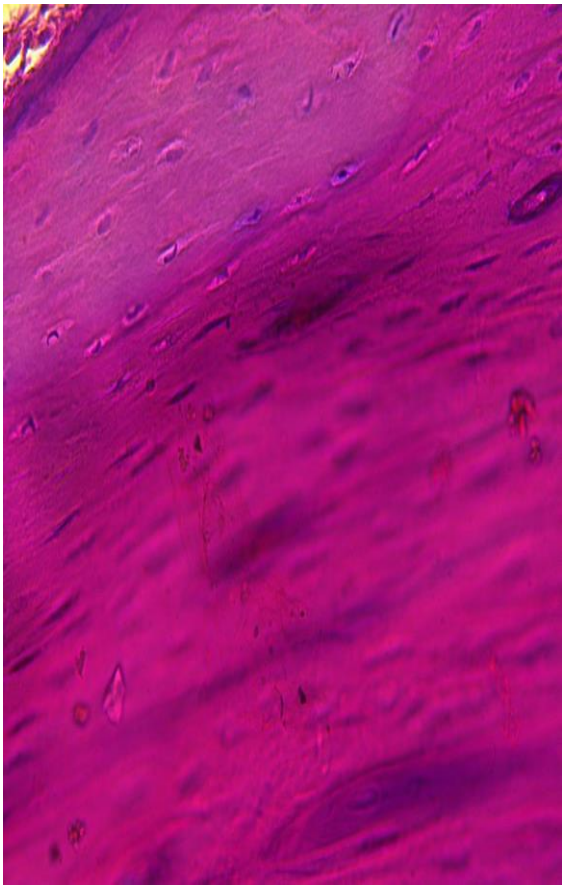

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Figure (9): The osteoid formation in biphasic calcium phosphate bone graft (Osteon II) group 3 after 2 weeks.

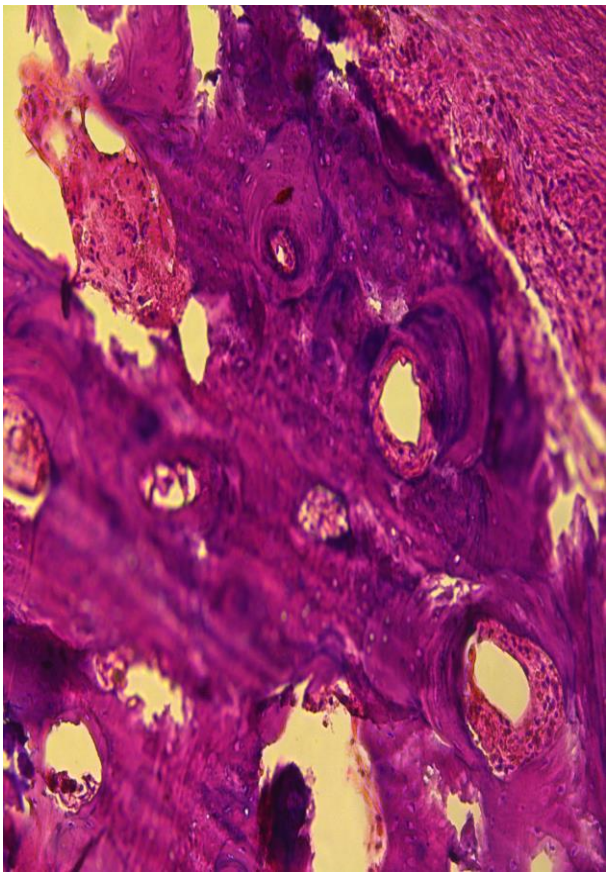

Figure (11): The bone formation in biphasic calcium phosphate bone graft (Osteon II) + collagen (hemospon) group 4 (2 weeks).

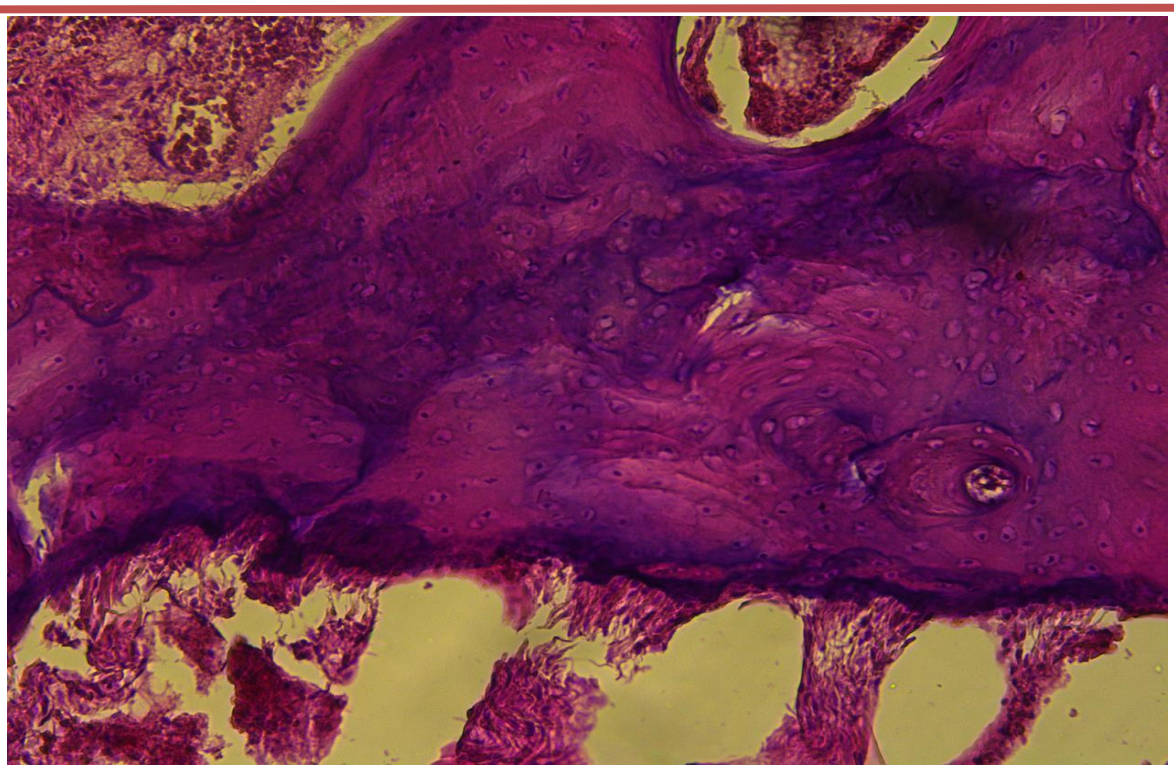

Figure (12): Low power view group4 (2 weeks).

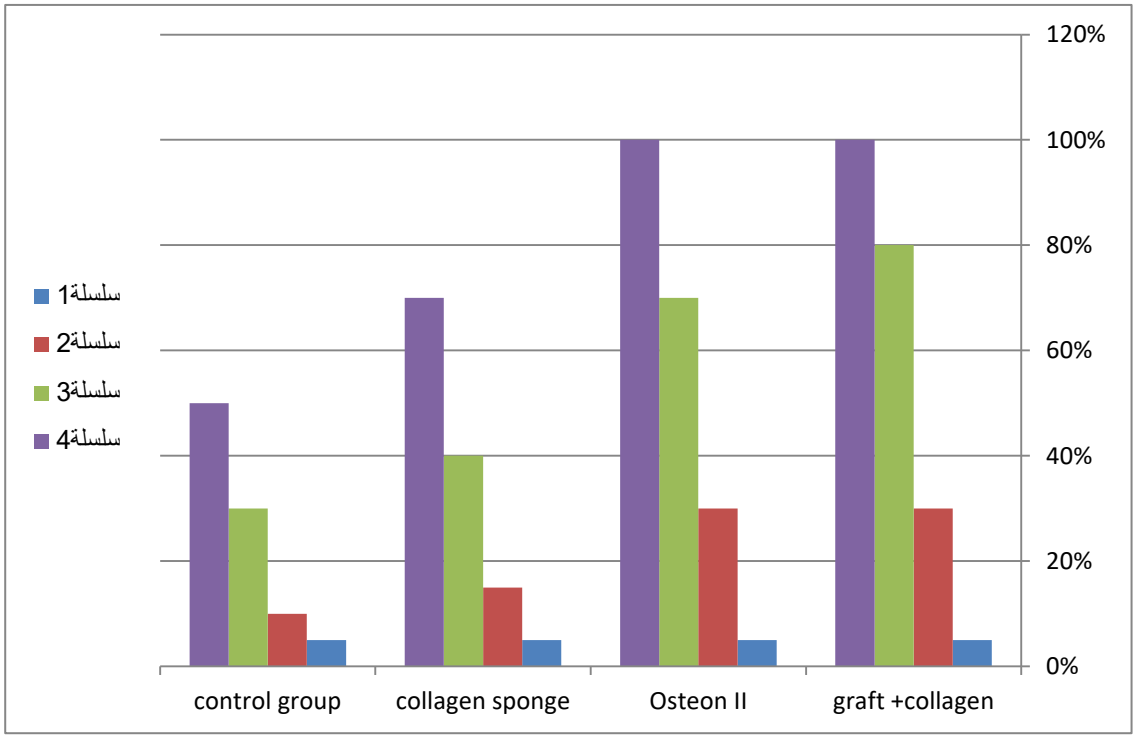

Figure (13): The healing % among the treated groups.

Table (1): The healing % for the treated animals by using the four different agents for 4 weeks.

Weeks	control group	collagen sponge	Osteon II	graft +collagen
1st	5%	5%	5%	5%
2nd	10%	15%	30%	30%
3rd	30%	40%	70%	80%
4th	50%	70%	100%	100%

Table (2):Chi- square test show that there is statistical significant differences between the four different groups.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	20.000[a]	24	.697
Likelihood Ratio	21.134	24	.631
Linear-by-Linear Association	2.036	1	.154
N of Valid Cases	16		

Table (3): show analysis of variance for the effect of time factor on the complete formation of bone for the four different groups.

ANOVA					
weeks					
s.v.	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13604.688	3	4534.896	14.250	.000
Within Groups	3818.750	12	318.229	Sig.	
Total	17423.438	15			

Table(4):show the mean healing period in weeks.

Report			
weeks			
Weeks	Mean	N	Std. Deviation
1.00	5.0000	4	.00000
2.00	21.2500	4	10.30776
3.00	55.0000	4	23.80476
4.00	80.0000	4	24.49490
Total	40.3125	16	34.08170

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