Iliac Crest Graft and Hydroxyapatite/ Β Tri- Calcium Phosphate for Repairing of Femoral Fracture: an Experimental Study

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Abstract

The present work was conducted for evaluation of the effects of iliac crest autograft (ICBG) and Hydroxyapatite / β tri- calcium phosphate (HA / β -TCP), on the healing process of an experimentally induced right femoral fractures with 0.5 cm × 0.5 cm partial bone defect at the fracture site in dogs fixed with standard dynamic compression plate (DCP) and self taping cortical screws. It was carried out on twenty seven apparently healthy male mongrel dogs divided into three equal groups, each of 9 dogs. Group I left as a control. Groups II and III were treated with ICBG and HA / β -TCP respectively. Clinical, radiographical and histological examinations were carried out for judgment of the healing process. This study came to the conclusion of; ICBG provides the most dense callus formation and the fastest possible healing. While, HA / β -TCP was an excellent substitute for ICGB that solve the problem of two operations on the same animal.

Introduction

Femur fractures usually require some sort of internal fixation. Fracture repair achieved by rigid fixation and perfect alignment of the bone to allow both timely and maximized return to function of the affected area ***(Baele, 2004)***.

There are some materials which stimulate bone healing such as bone autograft and synthetic materials (as ceramics based bone graft substitutes) are the choices in clinics to stimulate bone healing in orthopedic, spinal, and dental surgeries ***(Parikh, 2002)***. Autologous bone graft possesses osteoconductive, osteogenic and osteoinductive characteristics **(*Samartzis et al., 2005)***. Autogenous cancellous bone graft referred to be more osteogenic rather than cortical bone graft due to the presence of cavities in their structure permits the diffusion of nutrients and limited revascularization by micro-anastomosis of their circulating vessels ***(Hernigou et al., 2005)***. Autologous cancellous bone is usually harvested from iliac crest, the distal part of the radius and tibia, proximal humerus and distal femur with minimal morbidity and is transferred loosely to the fracture site. It is commonly used for delayed union, long bone defects and reconstruction of depressed fracture ***(Millis and Martinez, 2003 and Panagiotis, 2005)***.

Bone-graft substitutes can either substitute autologous bone graft or expand an existing amount of autologous bone graft ***(Jennifer et al., 2012)***. From a varity of ceramic based graft substitute materials, calcium phosphate based ceramics such as hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) is used effectively for long time in fracture healing ***(Laurencin et al., 2006)***. ***Chandrashekar and Saxena (2009)*** noted that, the new combination of synthetic hydroxyapatite and b-tricalcium phosphate are biocompatible and results in improved healing outcomes when compared with other calcium phosphate ceramic materials. These outcomes involve increase in the clinical attachment level and amount of bone filling the defects.

This study aimed to evaluate the efficacy of iliac crest graft and hydroxyapatite / β tri- calcium phosphate for acceleration of bone healing based on clinical, radiographical and histopathological examinations.

Materials and Methods

This study was carried out on 27 mongrel healthy adult male dogs ranged between 12 -18 months of age; at the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Suez Canal University. Food was withheld 6-8 hrs before the operation. Each dog was premedicated with I/M injection of chloropromazine hydrochloride (Misr Co. Pharm. Industries, S.A.A.) in a dose of 1mg /kg. The site of operation (lateral aspect of the right thigh) was aseptically prepared, and then general anesthesia was conducted by I/V injection of thiopental sodium 2.5% solution (Sandoz GmbH, Kund, Austria) until the main reflexes were disappeared. The right femur of all animals was experimentally fractured transversally by using a wire saw in the mid-diaphysis under constant cooling with a 0.9% NaCl solution with Cefaxone (*Pharco B International. Alexandria, Egypt*) (Fig. 1).

The dogs in this study were divided into 3 equal groups after creation of a 0.5 cm × 0.5 cm partial bone defect in the near cortex at the induced fracture site. The induced femoral fracture was fixed in all groups by DCP and self taping cortical screws. Group I, left as control (Fig. 2), while in group II, the gap was packed with iliac crest autologus bone graft (Fig. 3 A& B). In group III, the gap was packed with BioGraft-HT ;70% synthetic Hydroxyapatite and 30% β tri-calcium phosphate granules, manufactured by *IFGL refractories Ltd.* (Fig. 4).

Dogs were clinically examined daily during the study period (12 weeks). The functional disorders of the affected limb during walking were evaluated according to ***Fox et al. (1995)*** (Table 1).The data was recorded during the 1^{st}, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th} weeks after surgery.

Radiographs were taken on the 4^{th}, 8^{th} and 12^{th} weeks postoperatively.

For histobathological examination, the bone specimens were obtained from the fractured seat in all operated animals on the 4^{th}, 8^{th} and 12^{th} weeks postoperative after euthanasia of dogs using overdose of thiopental sodium. The bone specimens were prepared according to ***Hobbenaghi et al. (2014)***.

Data of the present study were analyzed using One-way Analysis of Variance (ANOVA) procedures according to ***(Snedecor and Cochran, 1989)*** for testing of significance between the studied groups. Means separation and pair wise comparisons were done by Duncan's Multiple Range test according to ***(Duncan, 1955)***. Statistical analyses were conducted by SPSS for windows (SPSS version 20). Results are considered significant at probability level of 0.05 for each ($P \leq 0.05$).

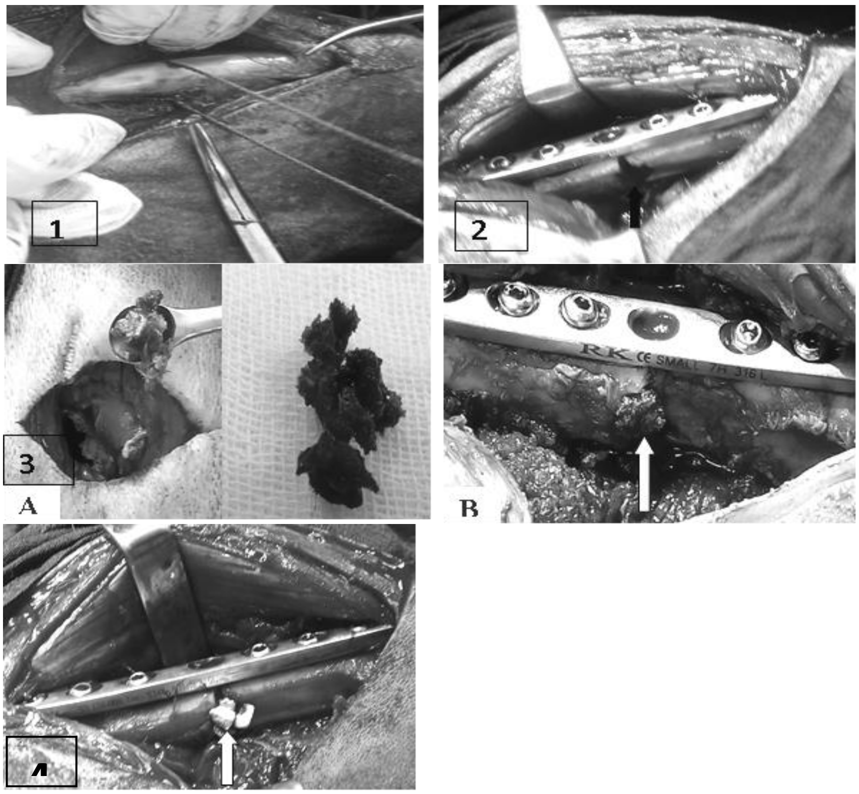


Fig. (1): Showing induction of femur fracture using a wire saw
Fig. (2): Showing fixation of the fractured bone with DCP and self taping cortical screws and the gap created at the fracture site (arrow).
Fig. (3): A. Showing harvesting the iliac crest cancellous autograft bone graft **B**. Showing packing of the harvested autologus bone graft (yellow arrow) into the induced fracture gap
Fig. (4): Showing packing of Biograft-HT (yellow arrow) into the induced fracture gap.

Table 1: *Scales of walking during the convalescent period according to* ***Fox et al. (1995)***.

Degree	Description
0	Normal attitude in station and walking without lameness.
1	Difficulties in walking, especially at rapid carriage with fine lameness.
2	Difficulties in walking, intermittent lameness in rapid walking.
3	Evident lameness at every step.
4	The leg pulls out of support in station and in walking, intense pain.

Results

In the first day after surgery lameness was pronounced in all dogs beside the local inflammatory signs (pain, redness, hotness, swelling and tenderness) which were gradually decreased till complete disappearance on the 6th postoperative day in all dogs. Dogs of groups I and III showed marked improvement in the mean degree of lameness towered the end of the first week postoperatively with partial weight bearing. Dogs of group II still revealing scale 4 of lameness, where the legs pulled out of support in station and walking with dragging of the hind limb. On the 2nd postoperative week dogs of groups I and III showed intermittent lameness during trotting, while lameness was evident at every step in dogs of group II. On the 4th postoperative week dogs of all groups showed further improvement in weight bearing with a convincing leg usage. By the end of the 6th week after surgery dogs of all groups showed fine lameness at rapid carriage. The least degree of lameness during the study period with functional leg usage was noticed in dogs of group III three months postoperative. There is no significant difference between group I and group II in the total mean of lameness. There is a significant increase in the mean degree of lameness in group II compared to group III (Fig. 5).

Radiographs showed a mild radiopaque periosteal reaction in groups I and III which become denser in group II and a radiolucent fracture line in all groups on the 4th week postoperative. On the 8th

week, the radiopaque periosteal callus as well as radiopaque endosteal callus was found to bridge between the two fractured ends of the bone in dogs of group I (Fig. 6). In group II, radiographs clarified the cessation of the periosteal reaction and the bone begun to restore its normal architecture with disappearance of the fracture line (Fig. 7). Radiographs of group III showed faint radiolucent fracture line and normal contour of the bone. On the 12th week, Radiographic films of group I showed that, the radiopaque periosteal and endosteal reactions were found to be progressed with declination of the fracture line. The bone completely regains its normal contour with complete disappearance of the fracture line in animals of group II and the radiopaque external callus was completely resorbed. Radiographs of group III revealed very faint fracture line with remnants of the external callus (Fig. 8).

On the 4th week postoperative, microscopic examination of group I revealed a large endosteal callus composed of fairly arranged fibrous connective tissue with many newly formed capillaries and some inflammatory cells (Fig.9). Focal areas of cartilage and woven bone dispersed throughout the callus were noticed in group II (Fig. 10). In group III the fracture gap was impacted with regularly arranged dense connective tissue. On the 8th week, group I showed mature granulation tissue with collagen fibers on the internal surface of the bone at the fracture site. In group II, The fracture gap was filled with small bony trabecular that revealed areas of fusion with large cartilaginous centers (Figs. 11). Bridging of the fracture site with large cartilaginous areas was noticed in group III with absence of inflammatory cells (Fig. 12). On the 12th week, all of defect spaces in group I occupied by extensive fibrous tissue and small foci of separate bone lamellae found irregularly inbetween and the bone marrow showed mild regeneration signs. In Group II, revealed well developed mature compact bone anastomosing each other at the fracture gap with a moderately regenerated marrow spaces inbetween indicating signs of complete healing (Fig. 13). In group III, the fracture gap was occupied by large foci of moderately mature bone and the trabecular bone in the medullary cavity begin to anastomose with evidence of hydroxyapatite / β- tricalcium phosphate material that requires more time for resorption (Fig. 14).

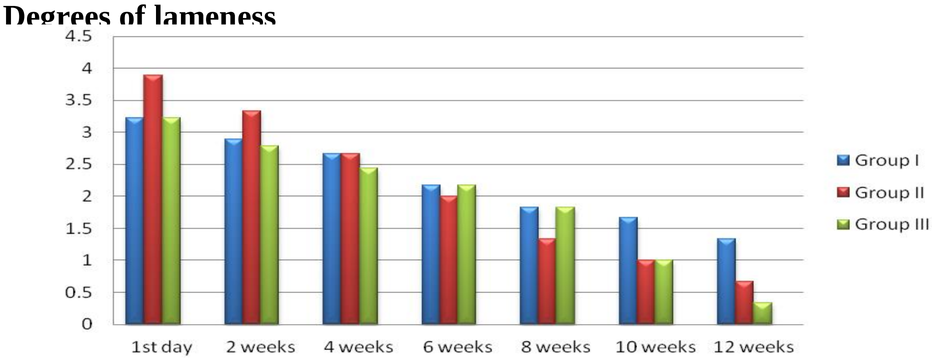
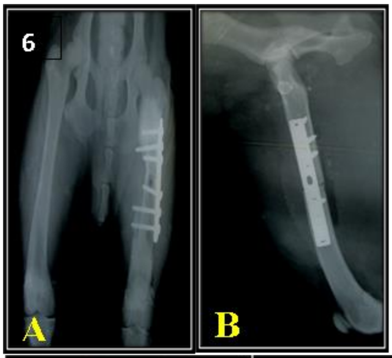
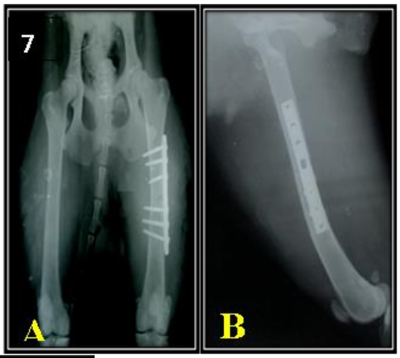


Fig. (5): Degree of lameness (mean ranks ± SE at different times among the experimental groups).





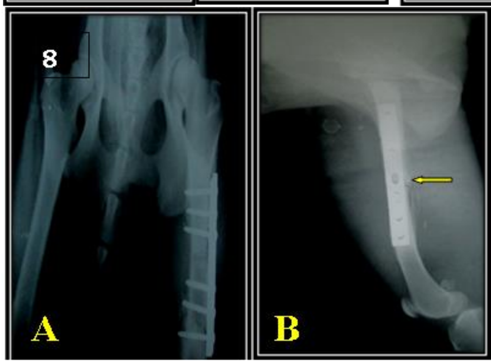


Fig. (6): Antero-posterior (A) and lateral (B) radiographic views in a dog of group I, 8 weeks postoperatively showing both radiopaque periosteal and endosteal callus bridging the fracture site.

Fig. (7): Antero-posterior (A) and lateral (B) radiographic views in a dog of group II, 8 weeks postoperatively showing disappearance of the fracture line.

Fig. (8): Antero-posterior (A) and lateral (B) radiographic views in a dog of group III, 12 weeks postoperatively showing very faint radiolucent fracture line with remnants of radiopaque external callus (arrow).

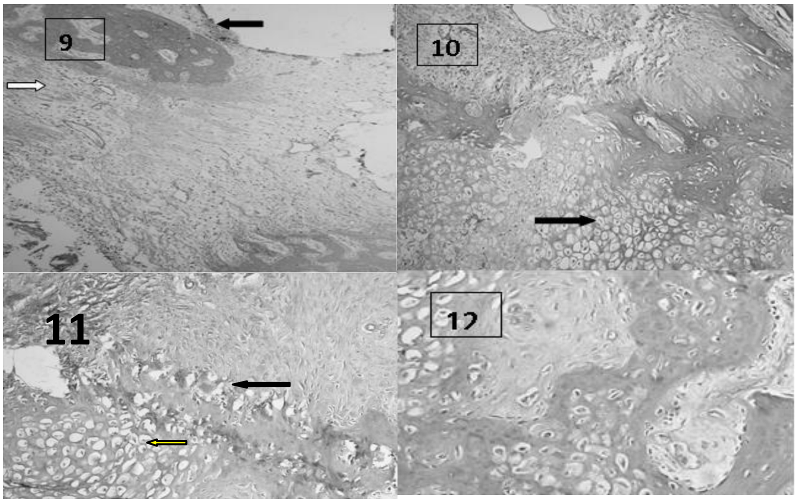


Fig. (9): Showing a large endosteal callus (yellow arrow) and small periosteal callus (black arrow). In a dog of group I, 4 weeks postoperative. H&E, X 40.
Fig. (10): Showing focal areas of cartilage (arrow) throughout the callus. In a dog of group II, 4 weeks postoperative. H&E, X100.
Fig. (11): Showing filled fracture gap with small bony trabecular (black arrow) with large cartilaginous centers (yellow arrow). In a dog of group II, 8 weeks postoperative. H&E, X 40.
Fig. (12): Showing large cartilaginous areas occupying the fracture gap. In a dog of group III, 8 weeks postoperative. H&E, X 100.

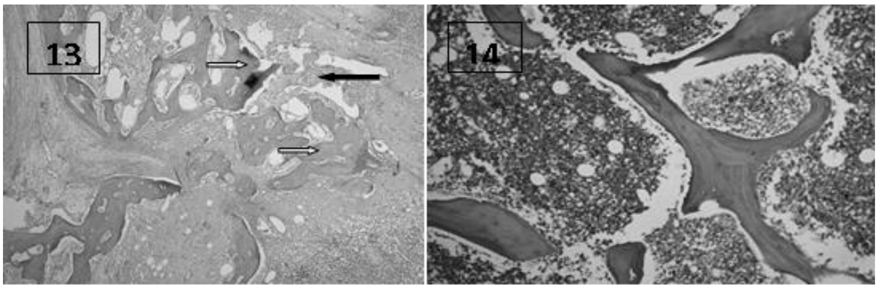


Fig. (13): Showing the trabecular bone in the medullary cavity begins to anastomose (yellow arrows) with evidence of hydroxyapatite / β- tricalcium phosphate (black arrow). In a dog of group III, 12 weeks postoperative H&E, X 40
Fig. (14): Showing mature compact bone anastomosing each other with a moderately regenerated marrow spaces. In a dog of group II, 12 weeks postoperative. H&E, X 100.

Discussion

Orthopedic surgery is a challenging and a rapidly progressed branch in veterinary practice. Regaining the normal function rapidly with the least complications is a demanded goal in orthopedic surgery as body movement based mainly on musculoskeletal system ***(Stefan and perren, 2002)***.

Exposure of the right femur was done by a skin incision on the lateral aspect of the thigh extending from the greater trochanter till the stifle joint. Similar approach was described by ***Denny and Butterworth (2000)***, who preferred this approach to avoid vital structures on the medial aspect and for the ease of manipulation of the femur. Revealing the femoral diaphysis was carried out by blunt dissection between the vastus lateralis and the biceps femoris muscles to minimize tissue damage. This was in agreement with ***Hulse and Hyman (1993)***, who reported that soft tissues around a fracture when minimally disrupted, it provide fragment vascularity and soft tissue envelope.

A wire saw was used to induce mid-shaft femoral fracture under irrigation with a 0.9% NaCl solution with antibiotic to reduce the heat released during sawing. ***Abass and Shekho (2009)*** used saline and antibiotic irrigation to decrease postoperative infection and to reduce the heat elaborated during sawing which intern lead to failure of fixation.

In the 1^{st} postoperative day, the local inflammatory signs were pronounced in all dogs which were gradually decreased till complete disappearance of inflammation on the 6^{th} postoperative day. Similar results were obtained by ***Madison and Martin (1993)***, who noticed that the inflammatory process reach the peak within 48 hrs after bone injury and completely disappear by one week post fracture.

Dragging of the hind limb (scale 4) was noticed during the first postoperative week in group II which become intermittent lameness during trotting (scale 3) on the 2^{nd} postoperative week due to perform two operation sites on the same limb. Similar results were recognized by ***Denny and Butterworth (2000)***. The mean degree of lameness decreased in animals treated with calcium hydroxyapatite/tricalcium phosphate compared to those of control group on the 4^{th}week. ***Chandrashekar and Saxena (2009)*** referred that this combination is biocompatible and resorbable in 6 months and presenting osteoconductive activity which enhance bone healing.

In radiography of control group, a clear fracture line with mild radiopaque periosteal callus was present on the 4^{th} week which began bridge the fracture line together with a large endosteal callus on the 8^{th} week. On the 12^{th} week, a declined fracture line with progressed periosteal and endosteal callus. These results were agreed with the results of ***Sande (1999)***.

The increased periosteal callus in this study may be attributed to the gap (0.5 cm × 0.5 cm with depth of the near cortex) induced at the fracture site. This was disagreed with those of ***McLaughlin (1991)***, who reported that healing with plate fixation is referred as primary which characterized by decrease or no relevant periosteal callus.

In autologous bone graft group, a dense radiopaque periosteal reaction with faint radiolucent fracture line was obvious on the 4^{th} week postoperatively which began to disappear at the 8^{th} week with complete disappearance of the fracture line at the 12^{th} week beside the resorption of the callus. These results were in agreement with ***Jones (1998) and Sande (1999)***, who observed that bridging callus of even density and uneven contour at 6-9 weeks. On 8-12weeks, dense callus of reduced size was present. Fracture line was hardly visible and early cortico-medullary remodeling was seen. On 10 weeks or more, further condensation of callus occurred, with distinct cortico-medullary separation due to remodeling and non visible fracture line was noticed.

In accordance with ***Eriksson et al. (1985)***, a new fracture was noticed distal to the end of the plate in a dog of group II on the 8^{th} week postoperatively. This was attributed to screws and drilled holes in the bone constitutes a stress risers that weaken the bone leading to fracture and refracture of the bone under bending conditions.

A faint fracture line and normal contour of the bone was noted at the 8^{th} week which gradually disappeared with remnants of the external callus towered the 12^{th} week postoperatively in group III. This result was supported by ***Yuan et al. (2002)***, who mentioned that calcium phosphate ceramics are hopeful synthetic bone replacement materials. They show good biocompatibility with bone due to their chemical similarities with bone mineral having benefits when used to accelerate bone formation.

The results of histopathologic examination on the 4^{th} week revealed a large endosteal callus covering the internal surface of the fractured bone with small periosteal callus in control group. In group II, the fracture gap was impacted with focal areas of cartilage and woven bone dispersed throughout the callus. Similarly ***Thor et al. (2007)***, reported that the fracture gap was wider in the control group rather than animals treated with bone graft (BG). Histopathologic examination for group III on the 4^{th} week revealed regularly arranged dense connective tissue that was meshed with newly formed capillaries and few numbers of inflammatory cells. Similar findings were obtained in the 5^{th} week by ***Servin et al. (2011)***. On the 8^{th} week, mature granulation tissue with collagen fibers on the internal and to some extent on the external surface of the bone found at the fracture site in control group. In iliac crest bone graft group, there was anastomosing thin bony trabeculae fusing the fractured parts with large cartilaginous centers. This result was matched with those of ***Thor et al. (2007)***, who demonstrated that the fibrous and cartilaginous tissues remained unmineralized in the control group, whereas the callus was nearly calcified in the treated group. Calcium hydroxyapatite/β tricalcium phosphate group on the 8th week showed bridging of the fracture site with large cartilaginous areas. These results were supported by ***Servin et al. (2011)***, who observed a cartilaginous center within the cortical bone with the absence of inflammatory cells.

Histopathological examination of control group on the 12th week revealed that, the fracture gap was filled also with small foci of separate bone lamellae and the bone marrow showed mild regeneration signs. On contrary, groups treated with iliac crest bone graft and Biograft- HT revealed complete healing on the 12th week which was evident by normal thickened cortex and the medullary cavity was also retained its normal anastomosing trabiculea. These results were agreed with those of ***Jochymeka and Gala (2007)***, who stated that the last signs of bony union are the redevelopment of the normal trabecular pattern and restoration of the continuity of the medullary cavity and cortex.

This study revealed that iliac crest bone graft is still the gold standard of bone transplantation based on clinical, radiographical and histopathological studies. It is rapidely incorporated into the host site. ***Khan et al. (2005)*** explained this incorporation is due to its osteogenic properties, abundance of growth factors and the large surface area that provides for bone formation. The cells present in the donor graft are capable of responding to local stimuli and realizing growth factors which accelerate angiogenisis and bone formation.

In accordance with ***Jones (1998)***, cancellous iliac crest graft was used in this study based on their osteogenic properities, permits diffusion of nutrient through their cavities and easily revascularized and incorporated into the host site. On the other hand, ***Hernigou et al. (2005)***, mentioned that cortical bone graft have a low osteoinductive properties but provide more structural support.

Harvesting bone autograft from iliac crest provides a considerable amount of autogenous graft with limited donor site morbidity, rich source of progenitor cells and its ease of harvest. Similar results were obtained by ***Panagiotis (2005)***. A dissimilar remark was observed by ***Millis and Martinez (2003)***, who reported that cancellous graft is perfect space filler, but it does not offer enough structural support.

Calcium hydroxyapatite/tricalcium phosphate together was used in this study to promote bone healing with no need to perform two operation sites on the same animal but it was much costly. ***Ghosh et al. (2008)*** reported that hydroxyapatite alone has moderate to low solubility within the body. ***Chandrashekar and Saxena (2009)*** illustrated that this combination of porous crystalline material provides good osteoconductivity and resorbability.

This study came to the conclusion that, autogenous cancellous bone grafts still the gold standard for enhancing bone repair. The combination of hydroxyapatite/ β tricalcium phosphate acts as a substitute for autogenous bone grafting and solving the problem of two operations in for harvesting the iliac crest bone graft but it is much costly.

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الطعمة العظمية من عظام قمة الحرقفة والهيدروكسي اباتايت / بيتا تراي كالسيوم فوسفات لإلتئام كسور عظمة الفخذ: دراسة تجريبية

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أجريت هذه الدراسه على عدد سبع وعشرون من الكلاب الذكور البالغه السليمه ظاهريا قسمت على ثلاث مجموعات متساويه تحتوي كل مجموعه على 9 كلاب.تم إحداث كسر في منتصف عظمة الفخذ اليمنى بها بواسطه المنشار السلكي وعمل فجوة في موقع الكسر بواسطة مقراض العظم مع التثبيت الداخلي وتم تثبيت كسر عظمه الفخذ داخليا بإستخدام شريحة إنضغاط ديناميكية ومسامير قشرية ذاتية الربط.

المجموعه الأولى تركت كمجموعه ضابطه . وفي المجموعة الثانية (مجموعة الطعمه العظميه من عظام قمه الحرقفه): تم الحصول على طعمه عظميه من عظام قمه الحرقفه من نفس الكلب وملء الفجوة بها . المجموعة الثالثة (مجموعة الهيدروكسي اباتيت/ بيتا تراي كالسيوم فوسفات): تم ملء الفجوة بماده الهيدروكسي اباتيت/ بيتا تراي كالسيوم فوسفات. و قد أجري الفحص الظاهري والاشعه التشخيصيه والفحص النسيجي لكل الحالات لتقييم عملية الشفاء على مدار اثني عشر إسبوعا.

وقد أظهرت النتائج أن إستخدام الطعمه العظميه من قمه الحرقفه أعطت نتائج أفضل في إلتئام كسور الفخذ وان الهيدروكسي اباتايت /بيتا تراي كالسيوم فوسفات يستخدم كطعمة تعويضية بدلا من الطعمة العظمية ويجنب عمل جراحتين في نفس الحيوان ولكنها مكلفه جدا.