



## Evaluation of The Role of Hydroxyapatite Nano Gel as Filling Materials for Improving The Healing of Repaired Tibial Bone Defect In Dogs



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**A**LARGE size bone defect is a challenging problem in orthopedic. This trial focuses on investigating the effects of Hydroxyapatite Nano gel as filling materials for accelerating the healing of repaired tibial bone defects in dogs. The experiment included 18 healthy dogs which was randomized divided into two equal groups, 9 of each. The experimental bone defect was created at (2.5 by 0.7cm) in both groups. In the control group, the same bone piece is reimplanted at the experiment defect and fixed with Cerclage wire. In the second group, a deproteinized lamb rib at (2.4 by 0.6cm) was used to repair the bone defect, and the graft segment was fixed with Cerclage wire, and the repaired defect was supported with hydroxyapatite Nano gel. The animals were monitored clinically, grossly and radiologically on days 14, 30 and 60. The defect completely healed with no apparent changes on day 60 post-operatively. Radiographically, in the control group on day 60, there was a faint lucent line around the fixated bone segment with minimal cortical thickening and irregularities denoting late chronic periosteal reaction and callus formation and near complete healing with the surrounding area whereas in the treatment group, the callus formation was superior in comparison with control group and there are some cortical irregularities denoting chronic periosteal reaction. We demonstrate that using hydroxyapatite nano gel as an additive to the defective bone fills the voids, accelerates the healing process. Radiological analysis showed superior healing and callus formation in hydroxyapatite nano gel group with xenobone graft.

**Keywords:** Tibia defect, Nano Hydroxyapatite, Grafting process, Dog

### Introduction

The major bone defect (large size defect) is a critical condition in bone healing caused by intense trauma, congenital deformities, fracture, retard in union or disabling blood supply to the defect [1], if the diameter of bone defect length 1.5 times considers as critical size [2]. The extended bone loss in this defect directly influenced vascularization and tissue healing, which leads to retard in the union of bone edges [3]. The transplantation of bone to restore and reconstruct bone defects is now a routine surgical intervention. Many agents and materials have been used

including cancellous, cortical, corticocancellous or synthetic bone grafts [4] or Nanoscale silk-hydroxyapatite hydrogels as bioactive materials [5]. Grafting of the bone is the surgical procedure in which a piece of bone is transferred from a healthy animal's donor site to a recipient site to replace lost bone [6] which underwent trauma or congenital deformities lead to bone loss and to replace the loss we need the bone transference this graft is used to restore a segment of bone defect or to enhance the nonunion fracture. A bone graft can be obtained from the iliac crest, tibial bone, humerus bone, ribs and sternum. A perfect graft material should be a biologically inert, osteogenic

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source, act as a scaffold, available, easily adaptable in shape, size, length and substituted by the host bone [7]. Many bioactive materials are used for grafting as osteogenic substrates such as platelet rich plasma for repairing tendons [8] and on xenograft in peri-implant bone defects in rabbits [9] using Xeno-Sheep Bony Implantation in rabbits [10] and PRP in the healing of grafted bone defects and Achilles tendon in dogs [11-13]. Hydroxyapatite,  $[Ca_{10}(PO_4)_6(OH)_2]$ ; HA], has achieved significant application as a bone graft material in a range of medical [14] and veterinary applications, it may be natural from eggshell for repairing bone defect [15] or synthetic such as calcium phosphate based biomaterials (hydroxyapatite), are most widely used in the orthopedic surgery. This material is considered an ideal biological graft material [16]. It is used as filling materials for bone defects and the creation of the residual ridge. However, paste and other gelatinous bone-filling materials can easily be used to fill defects and have good operability, hence they also allow for soft-tissue augmentation to be performed simultaneously [5,17]. Hydroxyapatite has biocompatibility, bioactivity and osteoconduction, and these properties permit their implantation into the bone site without an immune response. This agent can bind directly to tissue bone, promoting osteogenesis and new bone formation along its surface [18]. Also, the hydroxyapatite with zeolite/collagen was used to repair the critical size defect in rabbit with superior value of the index of a union. [19]. We hypothesize that, hydroxyapatite Nano gel will contribute to the grafting process of bone graft in dogs.

Up to date there is not enough information about the application of hydroxyapatite Nano gel as filling materials in the grafting process of lamb rib xenograft for accelerating and improving of bone tissue defect healing.

### **Material and Methods**

Eighteen dogs of local breed their age and weight were  $2\pm 0.6$  years,  $19\pm 0.45$  kg, respectively, were included in this study. Experiment assigned in two equal groups 9 for each. A mixture of 10% Ketamine 5mg (Alfasan, Holland), 2% Xylazine (Alfasan, Holland) 5mg/kg BWt., respectively was used to anaesthetize animals [20]. The analgesic protocol included Flunixin Maglumine 1.1mg/kg intravenously every 24hours (Uvefluxin®-Uvedco/Jordan)[21]. The study was approved by the Committee Ethics

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for Animal Research of the University of Mosul, College of Veterinary Medicine, in code of ethics (UM.VET. 2020.01). The operative site just over the proximal part of the tibial bone was prepared under strict sterilization the skin and muscle incised and bone exposed. An experimental bone defect (2.5 by 0.7cm) was induced by an electrical saw (Pencil Grinder/Royce/China) at the medial aspect of the tibial bone in the dog in both groups. In the control group, the same bone piece is re-implanted at the experiment defect and fixed with cerclage sterile stainless the graft is fixed firmly with sterile stainless wire the underlying tissue and skin are closed routinely. In the second group, deproteinized lamb rib at (2.4 by 0.6cm) was used to repair the experimental bone defect, then Hydroxyapatite Nano gel as filling materials along the edge of the repaired defect. All operative animals were monitored clinically for lameness, infections dehiscence of the wound, gross and radiological evaluation on days 14, 30 and 60.

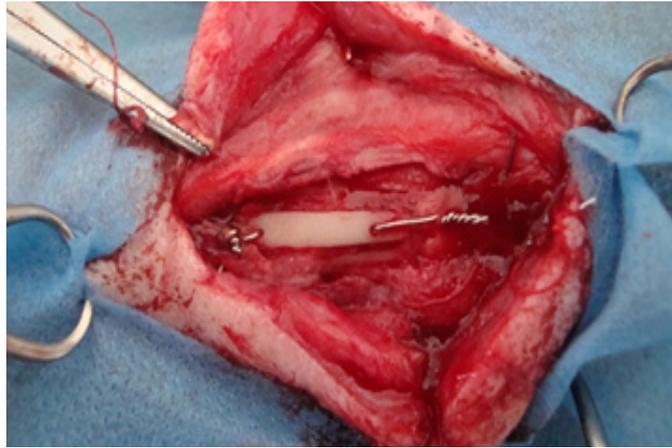
### **Results**

The outcome exhibited no dehiscence or signs of infection during the study period, and none of the animals died or developed undesired habits. Grossly, in the control group 15 days post operation the operative site demonstrated partial healing with signs of the inflammatory reaction of the soft tissue around the site of the operation (edema & congestion) (Fig. 1).

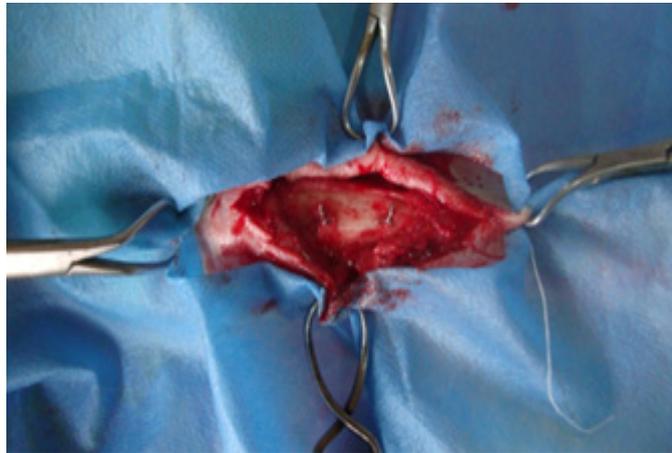
On day 30 post operation, the visual investigation exhibited no inflammatory reaction (Fig. 2). Whereas, on day 60 post operation also showed the bone with healthy appearance without any inflammatory signs (Fig. 3).

The visual examination of the treated group with hydroxyapatite Nano gel on day 15 showed an inflammatory reaction (edema & congestion) of the surrounding area with partial healing of the defective bone (Fig. 4). On day 30, there was complete healing of the defect edges with a slight inflammatory reaction (Fig. 5). The visual examination on day 60 showed complete healing and the bone appeared healthy with no inflammatory reaction Fig. (6).

The radiographic outcomes were as the followings: In the control group on day15 Lateral view (Leg): Status post fixation of the bone segment by cerclage wire in experimental bone defect at the upper tibia. There was a lucent line surrounding the fixated bone segment, associated with soft tissue swelling surrounding



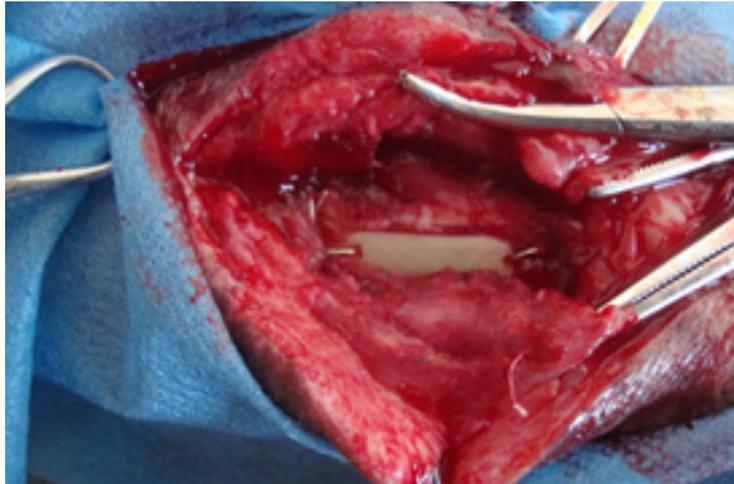
**Fig. 1.** Auto bone segment from same animals control group on day 15 post-surgery



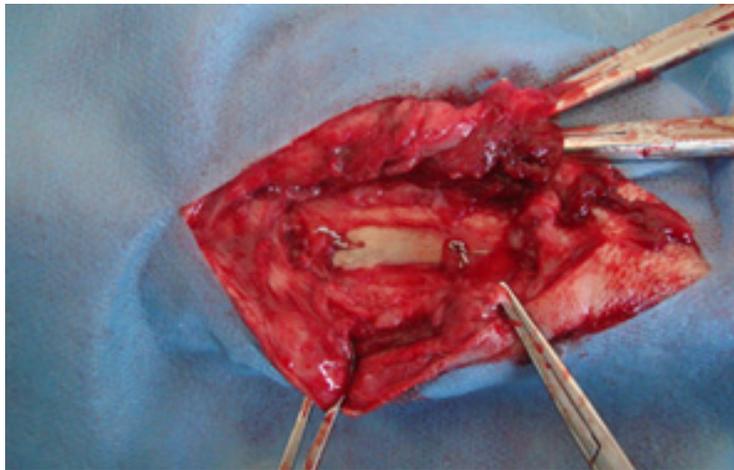
**Fig. 2.** Auto bone segment from same animals control group on day 30 post surgery



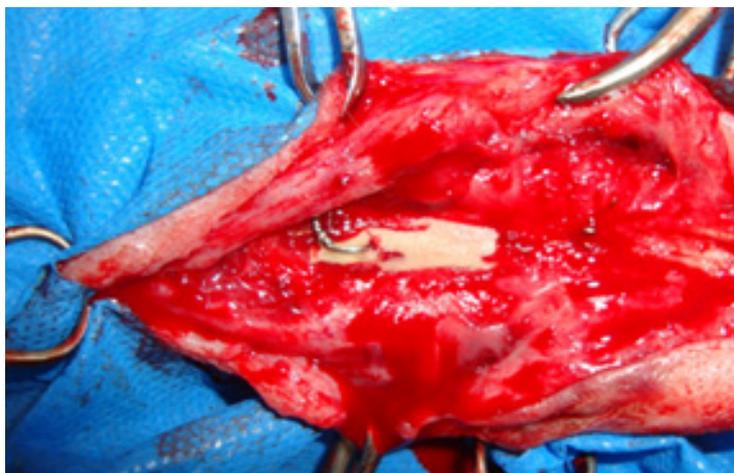
**Fig. 3.** Auto bone segment from same animals control group on day 60 post-surgery



**Fig. 4.** Hydroxy apatite Nano gel group second on day 15 post-surgery



**Fig. 5.** Hydroxy apatite nano gel group second on day 30 post-surgery



**Fig. 6.** Hydroxyapatite nano gel group second on day 60 post-surgery

the area, however no definite periosteal reaction nor significant callus formation (Fig. 7). On day 30 Lateral view (Leg), There was cortical irregularity and thickening with loss of the lucent line defect surrounding the fixated bone segment denoting late stage periosteal reaction with good callus formation and healing process (Fig. 8) whereas on day 60: There were faint lucent line around the fixated bone segment with minimal cortical thickening, irregularities denoting late chronic periosteal reaction, callus formation and near complete healing with the surrounding the area (Fig. 9). In the treatment group with Hydroxyapatite Nano gel on day 14 (oblique view), There was a lucent line all around the

fixated bone graft, associated with mild soft tissue swelling surrounding the area, however with some cortical irregularity and thickening denoting associated periosteal reaction but no significant callus formation (Fig.10). On day 30, there were minimal cortical irregularity and thickening with near complete loss of the lucent line defect surrounding the fixated bone segment denoting late-stage periosteal reaction but with good callus formation and ongoing near completed healing process (Fig. 11). On day 60 (oblique view), there were in progress healing of the bone segment with the surrounding area as crossing callus, however, some cortical irregularities denoting chronic periosteal reaction and callus formation (Fig.12).



**Fig. 7 . Radiographic image in control group on day 15 post surgery**



**Fig. 8 . Radiographic image in control group on day 30 post-surgery**



**Fig. 9.** Radiographic image in control group on day 60 post surgery



**Fig. 10.** Radiographic image in second group (Hydroxyapatite nano gel ) on day 14



**Fig. 11.** Radiographic image in second group (Hydroxyapatite Nano gel) on day 30



**Fig. 12. Radiographic image in second group(Hydroxyapatite Nano gel ) on day 60**

### **Discussion**

The animal models are the crucial procedure for testing the orthopaedic implants before their practice in humans. Many beneficial characters associated with using dogs as an experimental model in comparable to other animals, including their ability to tolerate different climatic conditions, easily adapts to the new environment and learn, as well as its cheaper availability [22]. Bone graft is categorized into several types: autograft, allograft, xenograft and bone graft alternatives. So, xenograft is now routinely performed with successful outcomes [23]. Many factors, such as the loading of the graft, the type of graft segment and the graft chips size may all influence the incorporation technique [24]. The perfect bone segment must be biocompatible osteogenic with structural support. In our study, we used a deproteinized xenograft of lamb ribs which fulfilled almost all achievement requirements of bone repairing, These results consider by some workers [19,25]. So, in the present study, bone graft acts as a scaffold restoring bone close to the natural form of defective tissue and acts as a bridge loading body weight and accelerating bone tissue healing in agreement with some authors [10]. Recently, bioactive agents have been used to provide osteoinductive properties such as hydroxyapatite [26]. Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is a major inorganic component of the bone that has been used extensively in many biomedical applications and bone regeneration due to its biodegradable, bioactive and osteoconductive properties [27].

This substance does not induce disadvantageous, local tissue reaction or immunogenicity and systemic reaction [28]. In this study, we used hydroxyapatite Nano gel with high viscosity (33%), which was prepared by strict sterilization and proceeded firmly and homogeneously spread along the edge of repaired bone defect, and this preparation proceeds as gel form could improve the practical utility of Hydroxyapatite this finding pointed by many investigators [5,28]. The fast mineralization of bone and acceleration in the healing process in the treatment group this due to the role of host blood vessels which makes a network easy to transport the osteoblastic cells to the site of defect this may be related to the osteoconductive properties of hydroxyapatite nano gel and this material are biodegradable, biocompatible with no any immune reaction [18,29]. Radiographic findings exhibited good knowledge about animals status. There was a close correlation between the radiological examination and clinical the treated animal return to normal activity and mechanical functions. There were also differences between the control and treated group. In the treatment group with hydroxyapatite nano gel progress healing of the bone segment indicated a zone of callus formations around the bone graft and complete healing with greater radiopacity [30]. In the first group, on day 30 post-surgery, a radiolucent edge gap with mild periosteal reaction was exhibited without bridging, and minimum callus around the repaired defect. In contrast, the second group demonstrated good periosteal reaction and callus formation

around the repaired defect. These outcomes may be related to using hydroxyapatite nano gel which enhanced osteoconduction and osteogenesis at the same time, which led to improvement in bone healing these results correlate with some researchers[31], who observed that the using crab shell-derived  $\text{CaCO}_3$  nanoparticles and natural polymers in the scaffolds help in the formation of new bone which acts as filling materials of bone gaps. However, despite beneficial results in the control group, the time to achieve total healing is still slow in progress healing of the bone segment with the surrounding area as crossing callus. In the hydroxyapatite nano gel group at 60<sup>th</sup> days the radiological investigations reveals chronic periosteal reaction and callus formation, the incorporation between defect ends and hydroxyapatite nano gel allowed be new bone tissue formation and the new bone tissue covering all space, this may related to osteoconductive properties of hydroxyapatite nano gel which permits osteoprogenitor cells to migrate across implanted segment this results agree with some researchers[32]. The using xenograft supported with hydroxyapatite nano gel has been advocated with success superior in comparison with the control group. The quality of bone union and healing significantly improves. This is evident from the results of the radiological examination, there is no rejection or immune response after using the HA material these findings agree with some authors[33]. The Bone diseases and fractures are important and painful diseases [34] Also using nano drugs to treat diseases can be a useful and effective solution[35,36].

### **Conclusion**

The findings exhibited mechanical stability of bone segment and good biological performance of hydroxyapatite nano gel. Radiological analysis of bone healing was shown to be improved by applying a combination of the nano hydroxyapatite gel and bone grafting by enhancing the repair of bones suffering from damage.

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### **Conflict of interest**

The research has no conflict of interests.

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#### Article Highlights

1. Hydroxyapatite Nano Gel accelerate regenerative process of bone graft
2. Xenograft fulfilled almost all achievement properties for bone repairing .
3. Hydroxyapatite Nano Gel used as filling bioactive materials and accelerated formation of bone bridges during bone regenerative process.

## تقييم دور هلام صغانر الهيدروكسي ايتيت والمستخدم بشكل حشوات في تحسين التئام عيب عظم القصبه المعالج في الكلاب

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<sup>٢</sup> فرع الامراض - كلية الطب البيطري - جامعة الموصل- العراق.

<sup>٣</sup> فرع الاشعه - كلية الطب - جامعة نينوى - العراق.

يعتبر العيب ذا الحجم الكبير للعظم من التحديات الكبيرة في مجال جراحة العظام. ركزت الدراسة الحالية على التحري على تأثير هلام صغانر الهيدروكسي ايتيت في تسريع التئام عيب عظم القصبه المعالج في الكلاب. شملت التجربه ١٨ كلبا سليما قسمت عشوائيا الى مجموعتين متساويتين كل واحده تضمنت ٩ كلاب تم اجراء عيب تجريبي بابعاد (٥, ٢-٧, ٥ سم) ولكلتا المجموعتين. مجموعة السيطرة تم اصلاح العيب باستخدام نفس قطعة العظم المقطوعه وتثبيتها بخيط الفولاذ. في المجموعة الثانية تم استخدام رقع مغايره مزالة البروتين من ضلع الحمل الصغير بقياس (٤, ٢-٦, ٤ سم) لاصلاح عيب العظم وتثبيته بخيط الفولاذ وتدعيمه بحشوات هلام صغانر الهيدروكسي ايتيت. تم متابعة الحيوانات سريريا وعيانيا وشعاعيا ولفترات ٤ و٣٠ و٦٠ يوما. تم شفاء العيب تماما وبدون اية تغييرات غير مرغوبه خلال ٦٠ يوما بعد العملية شعاعيا في مجموعة السيطرة بعد ٦٠ يوم بينت النتائج وجود خط خفيف خافت حول القطعة المثبتة مع تتخن بقشرة العظم غير منتظم والذي يشير الى حصول تفاعل سمحافي متأخر. مزمّن وتكون دشبذ حول منطقة العظم بينما تميزت المجموعة المعالجة عن مجموعة السيطرة بقشره عظم غير منتظمه مع التفاعل السمحافي المزمّن. وجدنا ان استخدام هلام صغانر الهيدروكسي ايتيت كحشوة مألثة تضاف لعيب العظم يسرع من عمليات الالتئام وتحليل الاشعه يشير الى التئام متفوق وتكوين دشبذ وعظم جديد بعد استخدام هلام صغانر الهيدروكسي ايتيت مع الرقع العظمية