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Evaluation the effect of high and low viscosity Nano-hydroxylapatite gel in repairing of an induced critical-size tibial bone defect in dogs: Radiolographical study

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ABSTRACT

Many types of bioactive materials are categorized as bone tissue substitutes for reconstruction and regeneration of bone defects, such as nano-hydroxyapatite. The objective of the present study was to radiologically evaluate the bone healing process in experimentally induced tibial defects in dogs treated with two different viscosity concentrations of nano-hydroxyapatite gel. Twelve adult, healthy Mongrel dogs were included. A critical size bone defect of 3–0.7 cm was induced surgically in the lateral border of the tibial bone of the right limb of all dogs enrolled in this study. The dogs were then categorized into two treatment groups: Group 1 (6 dogs): The defect was filled with prepared hydroxyapatite nanogel at a concentration of 33%, and Group 2 (6 dogs) hydroxyapatite nanogel at a concentration of 24% was used as a filling material. The healing process of the tibial defect and associated clinical and radiolographical findings were recorded in all studied groups at 30 and 60 days postoperatively. The results of the current study showed complete healing of the induced defect in the absence of any signs of pain or discomfort. Radiographically, there was an increase in radiographic density in the first group at 60 days. There is continuing healing in the late stage of the bone segment with the surrounding area and a crossing callus with cortical irregularities, denoting a chronic periosteal reaction and a good healing process. In the second group, the defect was completely filled with cortical thickening, which appears denser, denoting a periosteal reaction. In conclusion, using hydroxyapatite nanogel with high viscosity as a bone substitute contributed to progressing bone tissue regeneration with good callus formation and giving perfect mechanical support to defective bone.

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INTRODUCTION

The critical-size bone defect is defined as the defect that will not heal spontaneously by the normal healing process **(Jin-Young *et al.*, 2005).** The reconstruction of the critical size of the tibial defect represents a challenging issue in dogs. So, various grafts are used to optimize the regenerative treatment of tibial defects as lamb rib xenografts **(Mohammed *et al.*, 2022).** The reconstitution of defective bone, especially that which results from nonunion, trauma, tumour excision, and the surgical operation of prosthetic grafts, is classified as a problematic and challenging issue in the veterinary field **(Lyu *et al.*, 2013).** Different types of bone substitutes were used for the reconstruction of bone defects, such as prepared lamb ribs **(Gabriela *et al.*, 2020; Mohammed *et al.*, 2021),** fabricated carp shell **(Atiyah, 2018),** fabricated eggshell (Zebone et al., 2020), and demineralized bone matrix of caprine nature, which were used as a bone substitute for repairing an experimental bone defect of the tibial bone **(Felipe *et al.*, 2020).** The surgical intervention of bone usually includes bone dissection, bone grafting to optimize pain control, stability, and mobilization and to minimize dysfunction of the affected site (**Abid** and **Mukhatar, 2019).** The use of bovine bone morphogenic proteins (BMP) in fresh bovine bone obtained directly from the abattoir had a beneficial effect and significantly enhanced the bone defect healing process in the Caprine model **(Mistry and Mikos, 2005; Felipe *et al.*, 2020).** Recently, hydroxyapatite has played a crucial role in industrial, technological, and especially biotechnology due to the structural similarities between hydroxyapatite and the calcified tissue of vertebrates **(Felipe *et al.*, 2020).** It has biomedical uses as compensatory parts for the affected parts of the bone tissue as well as being used as catalysts in various reactions **(Zhu *et al.*, 2022).** Many osteo-conductive alternatives, such as coralline hydroxyapatite and tricalcium phosphate, were used alone as void fillers in critical bone defects or combined with biologically active osteoinductive and osteogenic substrates such as platelet-rich plasma (BMP) **(Huebner *et al.*, 2019, Felipe *et al.*, 2020).**

The ideal bone substitutes must be biocompatible, bioabsorbable, osteogenic, able to provide mechanical support, easy to use in practical applications, and low cost. A composite graft combines osteoinductive, osteoconductive, and osteogenic properties and is available in a variety of structures, including pellets of calcium sulphate, ceramics of calcium phosphate, and hydroxyapatite **(Lind and Bünger, 2001).**

The aim of this study is to radiologically evaluate the bone healing process in experimentally induced tibial defects in dogs treated with two different viscosity concentrations of nano-hydroxyapatite gel.

MATERIALS AND METHODS

Animals and design of study

Twelve healthy adult intact Mongerel dogs were included in the present. The mean age of all enrolled students was 12±0.2 months, weighing 15±0.4 kg. Before inclusion, all dogs underwent a complete physical and clinical examination to ensure that they were completely healthy and free from infectious, contagious, or musculoskeletal diseases. All dogs underwent the same management conditions, including accommodation, feeding, and housing, at the Veterinary Medicine College, University of Mosul, during the period of research from October 2022 to February 2023. This study was approved by the ethical committee of the Faculty of Veterinary Medicine, Mosul University, Iraq. All dogs were treated in accordance with guidelines established by the international and institutional Animal Care and Use Committee. The animals were divided randomly into two equal groups of six dogs each, according to the following:

Group 1: In this group, a critical size bone defect was induced at the lateral border of the right tibial bone (3 - 0.7cm) that was treated with hydroxyapatite nano-gel 33% (high viscosity) as a bone substitute.

Group 2: the same bone defect as the first group was induced in this group, and was filled with hydroxyapatite nanogel (24% viscosity) as a bone substitute.

Preparation of hydroxyapatite Nano gel Muco-adhesive gel Preparation

Hydroxyapatite Nano gel (25nm) was prepared in the College of Veterinary Medicine at the Pharmacology Laboratory, University of Baghdad, according to **(Tuğcu-Demiröz *et al.*, 2015; Marriot *et al.*, 2010).** 0.125 g of Carbopol was dispersed in distilled water at 6.25 (w/w) 2% by stirring at 800 rpm for 60 minutes and adjusted by adding a few dropwise of 10% sodium hydroxide (NaOH). The (hydroxyapatite Nano powder Ca5 (PO4)3OH)) of (Hualanchem Co., China) mixed until a transparent gel formed and the gel PH was adjusted to 4.5. Finally, the substance was sterilized using ethanol.

Surgical procedure

A critical-size bone defect was carried out under general anaesthesia. The enrolled dogs were routinely premedicated with 0.04 mg/kg Atropine sulphate (Atrovap, Vapco, Jordan) given subcutaneously, ketamine hydrochloride 10% (Rotexmedica, Germany), and xylazine hydrochloride (Interchemie, Holland) 2% given intramuscularly. (10,3 mg)/kg, respectively **(Green and Thurmon 1988).**

The defect was experimentally created at the lateral border of the tibial bone (3–0.7–0.7 cm) (Fig. 1), and the defect was filled completely with Nanohydroxy apatite gel at 33% or 24 % concentration according to its group (Fig. 2).

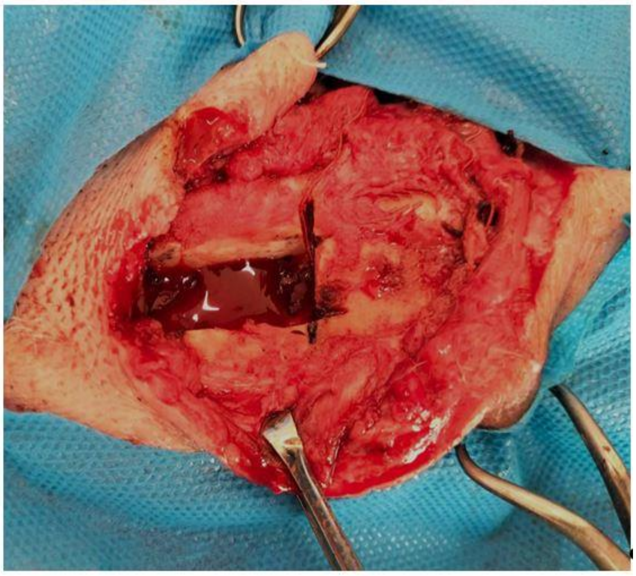

Fig.1: Represented the induced critical size tibial bone defect.

Fig. 2: Represented the Nanohydroxy apatite gel used as filling material

Clinical and radiographical evaluation

The animals were inspected daily along the period of operation form any abnormal signs as pain or discomfort. The radiographs (Shimadzu corporation-Japan) and digital radiography (DR-nexus -varex imagining corporation) of the defect of tibial bone were done at 30 and 60 days post-surgery using exposure factors of 60 kVp, 0.04 mA at 0.25 seconds and 90cm F.F.D in two views mediolateral, anterioposteriorly and at the right limb.

Post-operative follow

After treatment, OTC spray (O.L.KAR., Ukraine) was used directly on the operative site twice daily as wound care. Antibiotic (penicillin streptomycin) (Norbrouk, England) injected intramuscularly at a dose of 10000 IU and 10 mg/kg B.Wt, respectively for 3 consecutive days post-surgery.

RESULTS

The resulted data of present work shown that no any outward findings or any complications like seroma, severe hematoma, off food or bacterial inflammation were recorded along the period of experiment. The animals were kept alive during the two-months of experimental period.

Radiographically

In the first group: Status post insertion the Nano hydroxyl apatite 33% directly at the defective bone. There is minimal cortical thickening with hazy lucent line defect that surrounding the bone graft segment which appear narrow and dense denoting near complete crossing maturating callus formation and very good continuing healing process at 30 days post surgery (Fig. 3).

At 60 days there is continuing healing late stage of the fixated bone segment with the surrounding area and crossing callus with cortical irregularities denoting chronic periosteal reaction with good callus formation (Fig. 4).

Fig. 3: Radiographic image of healing progress of tibia defect using high viscosity 33% Nanohydroxy apatite gel at 30 days.

Fig. 4: Radiographic image of healing progress of tibia defect using high viscosity 33% Nanohydroxy apatite gel at 60 days

In second group, Nano hydroxy apatite 24% low viscosity lateral view at 30 days. There is haziness throughout the defect with minimal cortical thickening which appear more dense denoting periosteal reaction and early maturating callus formation and continuing healing process (Fig. 5). At 60 days. There is near complete filling of the defect with cortical thickening which appear more dense denoting periosteal reaction (Fig. 6).

Fig. 5: Radiographic image of healing progress of tibia defect using low viscosity 24% Nanohydroxy apatite gel at 30 days.

Fig. 6: Radiographic image of healing progress of tibia defect using low viscosity 24% Nanohydroxy apatite gel at 60 days.

DISCUSSION

Synthetic bone replacement materials act a crucial role in the treatment of critical bone defects. The osteo-regenerative and osteo-inductive properties of such substituted materials still need to be developed to obtain agents near autologous bone **(Dai *et al.*, 2015).**

The reconstitution critical defect of the tibia represented a challenge issue, especially in dogs **(Mohammed *et al.*, 2022).** The efficiency of nanohydroxyl apatite gel indicated good and faster healing in both concentrations. Last but not least, the animal study of using nanohydroxyl apatite as a filling material conjugated with bone graft exhibited a very good outcome without any complications **(Mohammed *et al.*, 2023).**

Radiological examination plays a crucial role in the characterization of the dynamics and nature of synthetic bone substitutes in orthopaedic surgery in veterinary medicine to obtain perfect union and bone integrity at the host bone along the bone regenerative process **(Arinzeh *et al.*, 2023).** The outcome of this study indicated that the use of hydroxyl apatite Nano gel at a concentration of 33% demonstrated that it is a safe bioactive material and that repairing and remodelling occur in the early stages of healing. Compared with hydroxyl apatite Nano gel at a concentration of 24%, there is ideal integrity between this material and the host bone, which these results agree with **(Mondal and Pal, 2019; Aoikanekoi *et al.*, 2020; Mohammed *et al.*, 2023).**

This study indicated that using nanohydroxy apatite gel highlights the improvement of fast generalization of induced bone defects and reduces the size of the induced bone gap. This agrees with the comparison of nanohydroxy apatite gel 30 and 20%, which were used at different concentrations with another bone substitute **(Juntavee *et al.*, 2021; Mohammed *et al.*, 2022; Ozawa and Suzuki, 2002).** The healing process completed during 8 weeks indicated that the nanohydroxyl apatite, especially in concentrations of 33%, represents perfect biocompatibility and is considered a good bone substitute agent for repairing tibia bone defects. This result also agrees with **Zheng *et al.*, (2004),** who suggested that the ossification was exhibited at 12 weeks post-treatment and new bone formation occurred.

CONCLUSION

In conclusion, using hydroxyapatite Nano gel with high viscosity as a bone substitute contributed to progressing bone tissue regeneration with good callus formation and giving perfect mechanical support to defective bone.

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Conflict of interest

The authors declare that no prospective conflicts of interest exist.

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