

NANOMATERIALS IMPLANTATION FOR ACCELERATING BONE HEALING

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ABSTRACT

The present study was conducted on 9 non-medicated, clinically healthy, adult mongrel male dogs. The dogs had no orthopedic abnormalities. Dogs were subjected to mid-diaphyseal circular bone defect (0.8 cm in diameter) in the left radius bones under general anesthesia. Dogs were divided randomly to be allocated into two groups, each of 3 dogs. The treated group (group T, n = 3), in which bone defects were implanted with the titanium oxide/graphene oxide/chitosan nanocomposite. The control group (group C, n = 3), in which bone defects were allowed for spontaneous healing. Dogs were subjected to clinical and radiographical evaluation 30 days postoperatively. All surgical procedures were conducted under the effect of total intravenous anesthesia (TIVA). Digital cranio-palmar and lateral views were taken for the operated limbs. Cortical defects and depth of the bone defects were recorded using the RadiAnt DICOM viewer version 1.1.2022 software. There was a significant decrease ($P < 0.05$) in the cortical defect in the treated group compared with the control groups 30 days postoperatively. The treated group recorded a significant decrease ($P < 0.05$) in 30 days compared to the baseline value. The depth of the bone defects decreased significantly ($P < 0.05$) in the treated group compared with the untreated group 30 days post-induction of the bone defects. There was a significant decrease ($P < 0.05$) in the treated group on 30 days compared to the baseline value. The titanium oxide/graphene oxide/chitosan nanocomposite accelerates the healing of bone defects.

Key words: Bone, defects, nanomaterials, healing, radiographic.

INTRODUCTION

Organic and inorganic components are put together in a highly complicated structure to

make up bone. When it is damaged, the entire body can no longer operate, which has an adverse effect on how the body moves and performs its many functions (Wang and Yeung, 2017). Bone grafting, or implant transplantation from one site to another, is one surgical treatment used to repair such injury. Allograft, xenograft, or autograft bone grafts are all possible. The most common

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treatment for bone injury is the autograft. Yet, due to its limited bone mass and handicapped structure, the autograft is less successful (Kheirallah and Almeshaly, 2016). The allograft may be a better option than autografting, which uses tissue from the donor's own body. Unfortunately, it commonly encounters some cases of immunology and disease (Venkatesan and Kim, 2010). The xenograft procedure involves implanting animal tissue into human tissue. Due to the significant danger of rabies and bone cancer, it is rarely done (Rossello *et al.*, 2013).

Much progress has been made in the treatment of bone tissue loss in recent years. This is performed by placing biodegradable materials into the bone defect site. These implants encourage the regrowth of bone tissue, which gradually deteriorates and is then replaced by newer bone tissue (Islam *et al.*, 2017).

There is a lack of studies regarding the use of titanium oxide, graphene oxide, and chitosan nanocomposite for the implantation of bone defects. This was the motivation to perform this study. Therefore, the present study aimed to evaluate the titanium oxide/graphene oxide/chitosan nanocomposite material for accelerating bone healing in dogs through clinical and radiographical evaluation.

MATERIALS AND METHODS

Ethical approval:

The present study was approved by The National Ethical Committee of the Faculty of

Veterinary Medicine, Assiut University, Assiut, Egypt.

Experimental animals:

The present study was conducted on 9 non-medicated, clinically healthy, adult mongrel male dogs. The dogs had no orthopedic abnormalities, 14 to 15 Kg body weight (BW), and were aged 2 - 3 years. Dogs were housed in standard cages with feed and water *ad libitum*. Dogs were subjected to mid-diaphyseal circular bone defect (0.8 cm in diameter) in the left radius bones under general anesthesia.

Experimental protocol:

Dogs were divided randomly to be allocated into two groups, each of 3 dogs. The treated group (group T, n = 3), in which bone defects were implanted with the titanium oxide/graphene oxide/chitosan nanocomposite. The control group (group C, n = 3), in which bone defects were allowed for spontaneous healing. Dogs were subjected to clinical and radiographical evaluation 30 days postoperatively.

Surgical procedures:

All surgical procedures were conducted under the effect of total intravenous anesthesia (TIVA) using 10 mg/kg/h ketamine 5% and 1 mg/kg/h xylazine 2% (Ibrahim, 2017). A circular bone defect (0.8 cm) was made at the mid-diaphysis of the radius using the bone drill according to Minto *et al.* (2015). The bone defects were implanted with the nanocomposite material in the treated group (n = 3). The bone defects were left for spontaneous healing in the control group (n = 3) (Figure 1A-B).

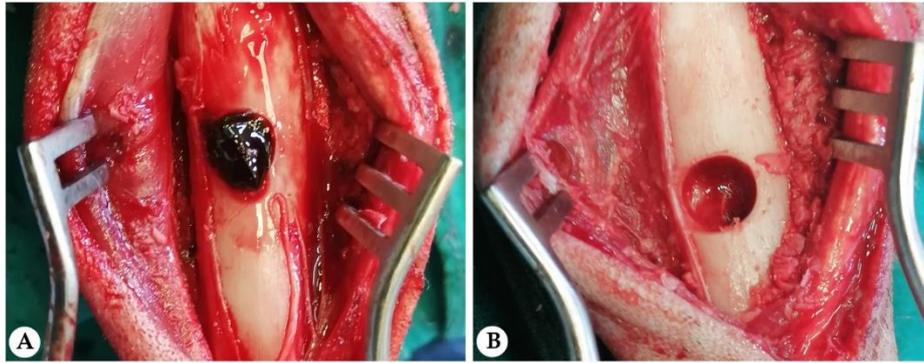


Figure 1: **A):** The bone defects were implanted with the nanocomposite material in the treated group. **B):** The bone defects were left for spontaneous healing in the control group.

Clinical evaluation:

Dogs were kept under clinical observation for 30 days.

Radiographical evaluation:

Digital cranio-palmar and lateral views were taken for the operated limbs. Cortical defect, and depth of the bone defects were recorded using the RadiAnt DICOM viewer version 1.1.2022 software.

Statistical analysis:

Data are represented as means \pm SE. Values with different small superscript letters in the same row for each parameter at different intervals are compared with time 0 and are significantly different ($P < 0.05$) using one-way ANOVA followed by Dunnett's post hoc test. Values with different large superscript letters in the same column for each parameter between treated and control groups are significantly different ($P < 0.05$) using unpaired t-test using GraphPad Prism software.

RESULTS

Table 1: The Cortical defect and depth of the bone defects in the treated and control groups

Item	Intervals (days)	0	30
Cortical defect	Treated	8.23 \pm 0.10 ^a	5.46 \pm 0.17 ^{b,A}
	Control (Untreated)	8.48 \pm 0.04 ^a	7.57 \pm 0.05 ^{a,B}
Depth	Treated	4.22 \pm 0.11 ^a	2.26 \pm 0.12 ^{b,A}
	Control (Untreated)	4.78 \pm 0.19 ^a	4.05 \pm 0.05 ^{a,B}

Clinical evaluation:

There were no recorded deaths or severe complications throughout the whole time of the study. By the end of the first week postoperatively, all dogs consumed their regular food.

Radiographical evaluation:

Cortical Defects:

There was a significant decrease ($P < 0.05$) in the treated group compared with the control group 30 days postoperatively. The treated group recorded a significant decrease ($P < 0.05$) in 30 days compared to the baseline value (Table 1).

Depth of the bone defect:

The depth of the bone defects decreased significantly ($P < 0.05$) in the treated group compared with the untreated group on 30 days post-induction of the bone defects. There was a significant decrease ($P < 0.05$) in the treated group on 30 days compared to the baseline value (Table 1).

DISCUSSION

Many clinical situations, such as fractures or orthopedic procedures, can lead to the development of bone abnormalities. Patients should have their normal bone structure restored as soon as possible. Autografts and allografts are two commonly utilized traditional ways of bone repair, although they have limitations of their own, the supply of materials for autografts is limited, and they run the risk of donor site morbidity, in some cases, using allografts might be preferable, but their use is constrained by the risk of an immune reaction and infection spread (Damien and Parsons, 1991).

Although their safety, effectiveness, and efficacy are yet unknown, a variety of synthetic bone substitutes composed of metal, ceramics, polymers, etc., have been created to speed up and improve the process of bone regeneration (Langstaff *et al.*, 2001). The last ten years have seen a substantial increase in the use of biomaterials to imitate the biological, mechanical, and structural characteristics of connective tissues (Silva *et al.*, 2020). Many of these materials demonstrated the ability to activate genes for cell differentiation, extracellular matrix development, and osteogenesis because they resembled bone (Pina *et al.*, 2015).

It has been noted that chitosan, a naturally occurring biopolymer produced from chitin, and several of its derivatives, including chitosan dihydrochloride, may function as bone substitutes, wound healing accelerators, antibacterial agents, and may exhibit hemostatic activity (Ezoddini-Ardakani *et al.*, 2012). The stability in body fluids, biocompatibility, minimal cytotoxicity, and mechanical properties (Chen and Selloni, 2014; Lee *et al.*, 2014). TiO₂'s ability to resist corrosion gives it great promise for application in bone implants (Fu and Mo, 2018; Garcia-Lobato *et al.*, 2019).

Many studies have shown that the materials in the graphene family, with their sharp

edges, functional chemical groups, and drug-synergistic effects, are capable of inhibiting the growth of bacteria. Moreover, it is difficult for the bone to successfully remodel and regenerate in an infected bone defect location (Cheng *et al.*, 2018). To the best of our knowledge, there is a lack of studies regarding the evaluation of titanium oxide/graphene oxide/chitosan nanocomposite implantation for accelerated bone healing, so the present study demonstrated its effectiveness on the rate of bone healing.

In radiographic findings, there was a significant decrease ($P < 0.05$) in the treated group compared with the control groups 30 days postoperatively, as well as a significant decrease ($P < 0.05$) in the treated group on 30 days compared to the baseline value. Depth of the bone defects significantly decreased ($P < 0.05$) in the treated group compared with the untreated group 30 days post-induction of the bone defects, with a significant decrease ($P < 0.05$) on 30 days compared to the baseline value. This may be attributed to the area under the grafted material gradually filled with a new bone formation, good periosteal reaction, and callus formation in the defect ongoing near the completed healing process. This was in disagreement with other researchers who reported that complete healing of such bone defect needs 60 to 90 days to complete healing (Arrigoni *et al.*, 2013; Calvo-Guirado *et al.*, 2014; Kim *et al.*, 2014; Guo *et al.*, 2018; Valencia-Llano *et al.*, 2021).

CONCLUSION

Titanium oxide, graphene oxide, and chitosan nanocomposite accelerate the healing of bone defects.

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زرع المواد الجزيئية لتسريع إلتئام العظام

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أجريت الدراسة الحالية على 9 كلاب ذكور غير معالجة، صحية سريريًا، هجين بالغ. لم يكن لدى الكلاب أي تشوهات في العظام. تعرضت الكلاب لنخر عظمي دائري متوسط الشكل (قطره 0.8 سم) في عظام نصف القطر الأيسر تحت التخدير العام. تم تقسيم الكلاب الي مجموعتين بشكل عشوائي، كل واحدة متكونه من 3 كلاب (n = 3)، المجموعة المعالجة Treated (حيث تم زرع النخر العظمي بأكسيد التيتانيوم / أكسيد الجرافين / مركب الشيتوسان) والمجموعة الضابطة (الغير معالجة) control group The (group C, n=3) (حيث سُمح بعيوب العظام للشفاء التلقائي بدون زرع للمركب). خضعت الكلاب للتقييم السريري والشعاعي بعد 30 يومًا من الجراحة. أجريت جميع العمليات الجراحية تحت تأثير التخدير الوريدي الكلي (TIVA). تم التقاط أفلام أشعة رقمية لجميع أماكن النخر العظمي بالرجل الأمامية بجميع المجموعات، تم تسجيل الخلل القشري وعمق عيوب العظام باستخدام برنامج RadiAnt DICOM viewer الإصدار 1.1.2022. أظهرت الدراسة انخفاض معنوي في الخلل القشري في المجموعة المعالجة ($P < 0.05$) مقارنة مع المجموعة الضابطة بعد 30 يوم من الجراحة. سجلت المجموعة المعالجة انخفاضاً معنوياً ($P < 0.05$) في 30 يوماً مقارنة بقيمة خط الأساس. انخفض عمق عيوب العظام معنوياً ($P < 0.05$) في المجموعة المعالجة مقارنة مع المجموعة الضابطة بعد 30 يوم من الجراحة. كان هناك انخفاض معنوي ($P < 0.05$) في المجموعة المعالجة خلال 30 يوماً مقارنة بقيمة خط الأساس. يسرع مركب أكسيد التيتانيوم/ أكسيد الجرافين / مركب الشيتوسان التئام عيوب العظام.