How to Cite:
Mohammed, S. M., & Rejeb, A. F. (2022). The use of nanoparticle form of milled teeth as a bone graft substitute in rabbits. *International Journal of Health Sciences*, *6*(S5), 6321–6332.
https://doi.org/10.53730/ijhs.v6nS5.10430

The use of nanoparticle form of milled teeth as a bone graft substitute in rabbits

Saber Mizher Mohammed
Master Student, Department of prosthetics Dentistry, Mosul University, College of Dentistry, Iraq
Email: saber.dep7@student.uomosul.edu.iq

Assistant Prof Dr. Atalla F Rejeb
Assist Professor, Department of prosthetics Dentistry, Mosul University, College of Dentistry, Iraq

***Abstract*---**Aims of the study: This study was designed to determine the effects of milled tooth in its nanoparticle forms as a bone substitute materials on the acceleration of bone healing in rabbitsMaterials and Methods: Sixteen albino rabbits were used in the experiment that were divided into two groups: nanoparticle group (M0 group) and control group. Rabbits were anesthetized using intramuscularly administered 10% ketamine (40mgkg) and 2% xylazine (5mgkg) (Inferchemi, Holland). After shaving and sterilization of the site of the operation, a 2cm submandibular incision was done. Then 3 millimeter circular anterior full-thickness defect was created near the midline of the mandible. The anterior defect was filled with 50-100nm milled tooth material nanoparticles. The incision was sutured with 3/0 black silk. Following surgery, each rabbit was given 30 mg/kg oxytetracycline intramuscularly. The rabbits were euthanized at 3, 7, 14, and 28 days afterwards. Then the specimens were dried and fixed in paraffin before being removed from the skulls. Histological examination the specimens was done. Results: The nanoparticle group(M0) showed moderate vascularity and granulation tissue formation, mild inflammation, and accumulation of osteoblasts over 3 days period, after 7 days period the findings were mild inflammation, new blood vessel formation formed inside new bone trabeculae and blood vessels in marrow space, mild granulation tissue formation and osteoblast accumulation for osteoid tissue formation. After 14 days period, in M0 group the findings were no significant inflammation, few viewed blood vessel formation, a moderate amount of granulation tissue formation, and mild to moderate osteoid tissue formation. Lastly, after 28 days M0 group showed no inflammation, good angiogenesis with new blood vessel formation, and few osteocyte lacunae formation with moderate osteoid

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 27 Feb 2022, Manuscript revised: 9 April 2022, Accepted for publication: 18 June 2022

tissue formation. Conclusions: The nanoparticles of milled tooth material treated groups are more stable and speed bone repair at various times (3, 7.14, and 28) days.

Keywords---bone substitutes, milled teeth, nanoparticle.

Introduction

In surgery, bone replacements are increasingly used. Every year, bone substitutes are used to create millions of bone transplants. To address the restrictions of using autografts, medical research has discovered other methods of bone healing **[1].** Allografts, xenograft replacements, and ceramic-based synthetic bone structures are all bone substitutes. The ceramic-based synthetic bone replacements are the most extensively employed in clinical operations. These ceramic bone replacements are working well **[2].**

The ideal bone replacement is a biocompatible composite. The use of a bone replacement has had harmful consequences in rare cases. The immune system is particularly sensitive to foreign entities and frequently assaults, then welcomes the graft. The body's response to transplants varies. Biocompatibility is the capacity of new bone replacements to quickly integrate into the body.

Traumatology, tumour surgery, spine surgery, infection, and revision arthroplasty all employ bone grafts. Annually, the US performs over 500,000 bone transplant treatments. These figures easily treble or triple globally, resulting in a scarcity of donor tissue utilised in bone repair treatments [3]. Autogenous bone graft has the highest osteogenic potential and is still regarded the gold standard for bone repair. New sources of autogenous cancellous bone from intramedullary canal are emerging. The RIA procedure was created to prepare long bones for intramedullary nail fixation **[4].** However, autogenous bone transplant surgery is a separate procedure, and harvesting difficulties have been recorded in up to 20% of cases **[5].** Easy usage, enhanced safety profiles, time savings, variety of sizes and forms, and minimal donor-site morbidity are all benefits of allograft. These are common alternatives to autogenous bone. Sterilization and storage alter the biological and mechanical characteristics, reducing osteoinduction and osteogenic potential. With increased demand for spinal fusion, revision arthroplasty, and joint fusion, allogeneic bone donors are in short supply. For these reasons, bone replacements are becoming more important **[3].**

Bone replacements vary in composition, mechanical strength, and biological function. Because each bone replacement has its own benefits and drawbacks, it is important to understand how biological features affect bone mending. This study will examine the qualities of bone grafts and other bone substitutes used in orthopaedic surgery **[6].**

Nanostructure

Nanotechnology is widely used in sectors such as medicine. Using nanotechnology, it is now possible to study and manipulate atoms, chemical bonds, and molecules in various substances. Nanotechnology is used in dental fields like nano dentistry. When choosing a nanoparticle for use in nano dentistry, its physical, chemical, and organic properties are considered. Adding atoms often affects the functional structure. Nanostructures are used in dental diagnostics. Only a few nanoparticles are used for dental implants and prostheses [7]. Nanotechnology is a technique that works with nanometer-sized particles called nanoparticles. Silver is the most widely used nanoparticle, followed by carbon and ion oxides (TiO_2) [8]. The nanotechnology may improve the product's features by introducing new functionalities. Thus, nano goods are widely used in many industries, medicine, and dentistry [9].

Bone substitutes and dental implant

Bone is a dynamic organ capable of regeneration. The bone-signaling route directs osteoblasts, osteocytes, and osteoclasts in the continuous cycle of bone production and resorption. Bone grafts and replacements are used when the host cycle of bone healing is insufficient. Bone grafts are second only to blood in terms of popularity. Worldwide, around 2.2 million bone-grafting surgeries are performed annually in orthopaedics, neurosurgery, and dentistry. More than 60% of the population in developed nations need dental prosthesis replacements, preferably with implants, and 10-20% of those individuals require bone regeneration operations prior to implant insertion. 80% of bone is solid (cortical), with 20% being spongy (trabecular). Thus, assessing compact bone composition will concentrate treatment on the bigger area of the bone. Bone graft design may concentrate on one compartment since compact bone is 70% inorganic mineral (hydroxyapatite), 22% organic protein (collagen, cells, HA) and 8% water **[10].**

Materials and Methods

Sixteen albino rabbits were used in the experiment that were divided into two groups: nanoparticle group (M0 group) and control group. Rabbits were anesthetized using intramuscularly administered 10% ketamine (40mgkg) and 2% xylazine (5mgkg) (Inferchemi, Holland). After shaving and sterilization of the site of the operation, a 2cm submandibular incision was done. Then 3 millimeter circular anterior full-thickness defect was created near the midline of the mandible. Rabbit's teeth were crushed into; (50 – 100nm) diameter as nanoparticles that were used in the nanoparticles group (M0 group), by Grind device used for milling animal teeth ground at the University of Technology in Baghdad, Iraq, as shown in Figure 1.

Figure1. Grind device used for milling teeth NQM-0.4 model planetary ball. (Yangzhou Nouya, China)

The study used sixteen adult male albino rabbits aged 5-7 months, weighing 1.5-2 kg. The animals at Mosul University's Animal House were fed green leaves, fruits, and water. A control group and a nanoparticle group were created. For group M0, a defect was created between the central and molar teeth of the lower jaw and filled with nanoparticles of milled teeth as a bone replacement. In the control group, one defect was produced between the central and molar teeth of each animal. The rabbits were caged outside and given greens, fruits, and vegetables. They looked to be in excellent health throughout the research period. All instruments were autoclaved. Milled tooth nanoparticles in pouches, ready for use.

Surgical procedures

Each animal was anaesthetized with 10% ketamine (40mg|kg) and 2% xylazine (5 mg|kg) intramuscularly (Inferchemie, Holland). Rabbit skin was shaved using clippers machines, and the operation site was sterilizedwith povidone-iodine. A 2 cm long incision was performed using a scalpel and blade no.12 (Medenta, China). A (2) cm long full thickness flap was mirrored by a periosteal elevator for bone exposure. A stainless steel bur drilled the bone. Making a hole defect for (M0 group) nanoparticles using a 2.5 mm bur as shown in Figure 2.

Figure 2. Shaving procedure, Incision procedure

Sample Study

The sample study is freshly extracted (16) slides, and divided into two groups control group and nanoparticles group (M0). (12) slides were used in Nanoparticles (M0 group). While (4) slides were used in the control group that was compared with the other samples. The whole slides for (M0) group were two slides for each rabbit.

Statistical Analysis

The data was examined using SPSS 21.0, an IBM programme. The study used descriptive and analytical statistics (t-Test). The differences between the three groups were statistically significant at $p < 0.05$.

Results

These the slides were separated into two groups of 3, 7, 14, and 28 days. Specimens were assessed histoologically using four criteria. These include the degree of inflammation, vascularity, granulation tissue, and osteoid formation.

Histopathological analysis for M0 group after three days

1. Inflammatory cells: The findings of microscopic examination of M0 group after three days observed that mild inflammatory cell infiltrates for all slides.
2. Vascularity: The findings of microscopic examination of M0 group after three days observed that a moderate amount of vascularity for all slides.
3. Granulation tissue: Moderate amount of granulation tissue was similar to all slides under microscopic examination.
4. Osteoid tissue formation: There was found accumulation of osteoblasts of osteoid tissue formation among the all slides after three days that showed under microscopic examination, as shown in Figure 3.

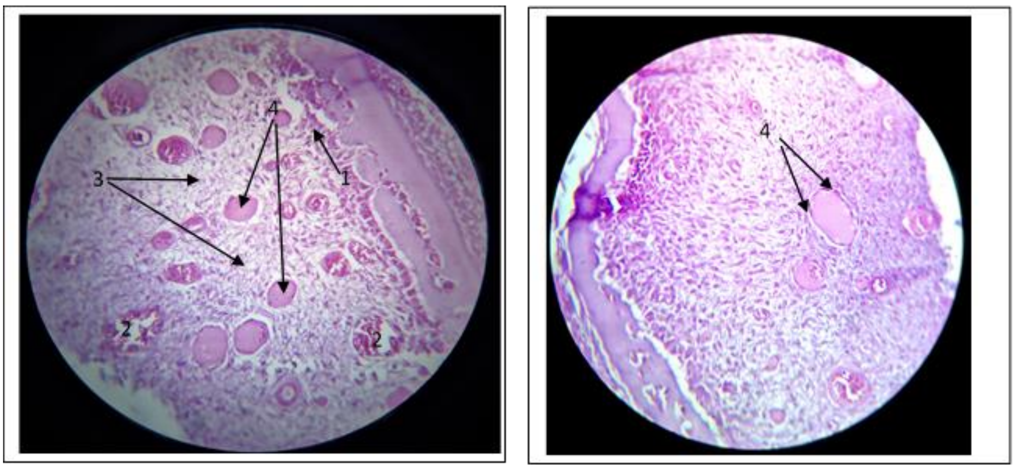


Figure 3. The defects of M0 group after three days.

Histopathological analysis for the control group after three days

1. Inflammatory cells: The findings of microscopic examination of the control negative group after three days observed that inflammatory cell infiltrate served.
2. Vascularity: The findings of microscopic examination of negative the control group after three days observed that no significant vascularity was found.
3. Granulation tissue: No significant amount of granulation tissue under microscopic examination was viewed.
4. Osteoid tissue formation: No significant amount of osteoid tissue formation was found after three days that showed under microscopic examination, as shown in Figure 4.

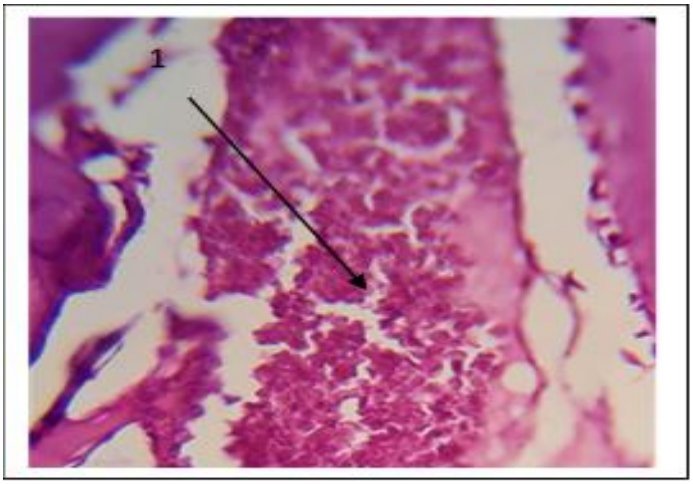
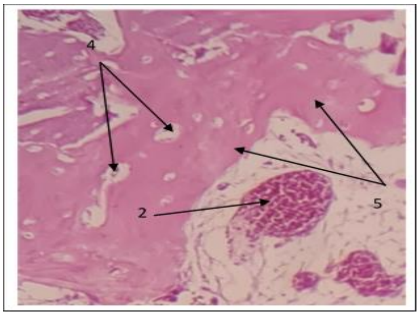


Figure 4. The defects of the control group after three days.

Histopathological analysis for M0 group after twenty eight days

1. Inflammatory cells: No significant inflammatory cell infiltrates were viewed under microscopic examination of M0 group after twenty eight days for all slides.
2. Vascularity: The findings of microscopic examination of M0 group after twenty eight days showed good angiogenesis with new blood vessels formation for all slides
3. Granulation tissue: Mild granulation tissue remained in the site of bone healing that was similar to all slides under microscopic examination.
4. Osteoid tissue formation: Accumulations of osteoblasts, few osteocyte lacuna formation and moderate osteoid tissue formation were seen among all slides after twenty eight days that showed under microscopic examination, as shown in Figure (5).



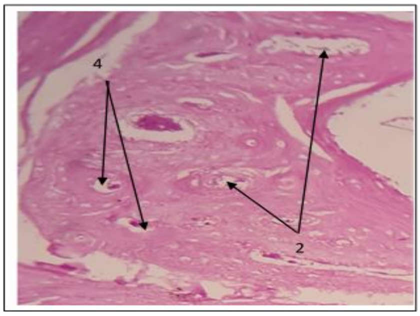


Figure 5. The defects of M0 group after twenty eight days.

Histopathological analysis for the control group after twenty eight days

1. Inflammatory cells: No significant of inflammatory cell infiltrates were viewed under microscopic examination after twenty eight days.
2. Vascularity: Good angiogenesis with new blood vessels formation were seen.
3. Granulation tissue: A moderate amount of granulation tissue remained in the site of bone healing under microscopic examination.
4. Osteoid tissue formation: Osteoblasts accumulation, few osteocyte lacuna formation and moderate amount of osteoid tissue formations were shown under microscopic examination, as seen in Figure 6.

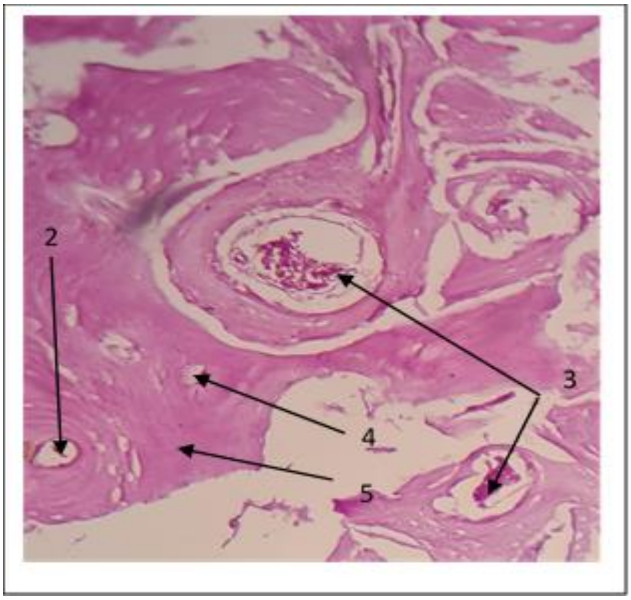


Figure 6. The defects of the control group after twenty eight days.

Histopathological and statistical analysis for different procedures with different times

The T-test was employed to analyse histology data. The little letters (a, b, c, d) denote substantial group differences. No significant difference within group P 0.05, and substantial difference within group P 0.05, respectively. The capital letters (A, B, C, D) reflect the significant difference between groups at P 0.05. Inflammatory cell responses: In the M0 treated group, inflammatory cell responses decreased significantly after 7, 14 and 28 days compared to 3 days in the same group. A significant decrease in inflammatory responses was seen in the M0 treated group compared to the control group after 7 days, and a similar decrease was observed at 14 days, as shown in Table 1.

Table 1. T-test for statistical analyses of inflammatory cell responses within and between M0 and control group at different times (3, 7, 14, 28) days.

groups	3 DAYS	7 DAYS	14 DAYS	28 DAYS
control	2±0.4 a A	2±0.4 a A	1±0.4 b A	0.2±0.1 a A
M0	2±0.3 a A	1±0.3 b B	0±0 c C	0.2±0.1 c A

The results regarding osteoid formation within the same group when compared at different times revealed a significant increase in control group at 7 and 28 days period comparing with 3 and 14 days period, while there was a significant increase of osteoid formation in the M0 group revealed a significant increase of osteoid formation after 14 and 28 days comparing with 3 and 7 days periods of same group.

The results of the criteria of osteoid formation in comparison among the groups showed no significant difference between the groups at 7 days period, but there was a significant increase of osteoid formation in the M0 treated group comparing with control group at 3 days period. In addition, there were a significant increase of osteoid formation in the M0 treated group comparing with control group at the 14 days period, as shown in table 2.

Table 2. T-test for statistical analyses of osteoid bone formation finding within and between M0 and control groups at 3,7,14, and 28 days.

groups	3 DAYS	7 DAYS	14 DAYS	28 DAYS
Control	0.2±0.1a A	1±0.4 b A	0±0 a A	2±0.4 c A
M0	1.8±0.2a B	1±0.3 a A	2±0.3 b C	3±0.5 c B

Discussion

Localized bone loss is dangerous. Finding an efficient local bone replacement offers several benefits and drawbacks [11]. In addition, bone replacements. Every year, millions of bone transplants occur. Other bone healing techniques have been developed to circumvent the limitations of autografts. The benefits of bone replacement include allografts, xenografts, and ceramic-based synthetic bone constructions [12].

1. We utilized rabbits for the research because they are docile and non-aggressive, making them simple to handle and watch.
2. Widely bred and cheap compared to bigger animals.
3. Short gestation, breastfeeding, and puberty cycles

4. It is a tiny animal and so falls within the jurisdiction of the local ethics commission. Larger animals need extra ethics committee approval, which is a lengthy and strict procedure.

It is very uncommon for big animal surgical operations to need complicated and costly rearing facilities that are only capable of central animal research institutions. Teeth and bones are related. They all sprang from the same place: the neural crest [13]. Within the mouth cavity, clinicians encourage intramembranous bone development [14]. Teeth, particularly dentin, and bones have similar chemical makeup. Enamel is 96% inorganic and 4% water, whereas dentin is 65% inorganic, 35% organic, and 4% water. Cement is composed of inorganic (45-55%), organic (50-55%) and water. Finally, alveolar bone is 65% inorganic and 35% organic. Dentine has been studied extensively owing to its high mineral concentration, which makes it a viable bone replacement. Dentine is also osseo-compatible and osteoconductive, like autogenous bone, providing a physical substrate for new bone development. This makes dentine a great bioactive material for hard tissue regeneration [15].

Even though it must be harvested, autogenous bone is still regarded the gold standard due to its osteoinductive, osteoconductive, and osteogenic qualities [15, 16]. claim that dentine is composed of the same elements as bone. Its organic matrix contains non-collagen proteins such phosphoproteins, osteocalcin, proteoglycans, and glycoproteins. So we utilized milled teeth to restore bone loss in rabbits' lower jaw. Materials nanoparticles (M0) replaced bone [17]. These smart features are advantageous for the creation of bone substitutes and platforms for bone tissue repair and regeneration. The present histological study slides. Each slide has three M0 and control groups with varying times (3, 7, 14, and 28 days). The histological examination of M0 and control specimens included four criteria. Oncoid development and inflammation were the criteria.

Inflammatory cell responses

In the M0 treated group a significant decrease in inflammatory responses was found after 7, 14, and 28 day periods as compared to 3 days at ($P \leq 0.05$). For the comparison among the groups, no statistically significant difference was found between the groups at 3 and 28 days periods for all groups. As related to the degree of inflammatory cell infiltrates; the M0, and control groups have shown similar behavior for all groups at 3 and 28 days periods. M0 treated group showed the lowest inflammatory cell response. Moreover, M0 treated group has a lower value of t-test compared with the control group over 7 days period.

Vascularity and the granulation tissue formation

At 3 days period the results of microscopic examination revealed that the M0 group a moderate vascularity and granulation tissue formation was shown. This might be due to nanoparticle size undergoing rapid resorption that attempted to heal faster than macroparticles. In the control group, there's no vascularity and granulation tissue formation was seen at 3 days period. The M0 group showed newly formed blood vessels inside new bone trabeculae and there was mild granular tissue formation and the control group showed little vascularity. At 14 days M0 group showed few blood vessels viewed which means they had healed. Lastly, at 28 days period, the control and M0 groups had good angiogenesis with new blood vessels formation which means all wounds were healed optimally without any rejection and no antigenicity reactions were seen.

Osteoid tissue formation

The M0 treated group revealed a significant increase of osteoid formation after 14 and 28 days compared with 3 and 7 days periods of the same group. Comparative analyses of the criteria of osteoid formation among the groups displayed no significant difference for all groups during 7 days period, however, there was a significant increase of osteoid formation in the M0 treated group compared to the control groups during 3 days period. Furthermore, M0 were more responsive to osteoid tissue formation compared with the control group during the 14 days. In the same context, M0 treated group showed more response to osteoid tissue formation compared with control group during the 28 days. These variations of rapid response at different periods occurred due to the effects of the type of nanoparticles for a bone substitute were used.

The ideal bone replacement should be biocompatible, have great osteoconductive properties, be strong enough to produce a desirable shape, and transform the bone fully in a short period. The materials (M0) are biocompatible and utilised as bone replacements. It was accepted with the findings in [18]. Unlike other research **Aroni et al., [19],** that employed various materials such deproteinized bovine bone (DBB) particles, this study allowed nanoparticles to provide a framework for new bone development. Moreover, grafting DBB with directed tissue regeneration slows bone growth **[20].**

Conclusions

Nanoparticles (M0) treated groups may replace bone. The M0 treated group has more stable osteoid tissue formation over time. It increased rapidly at 7 and 14 days for all groups, independent of inflammatory cell response, and then declined progressively. All groups had the greatest rise in bone replacement at 14 and 28 days, independent of osteoid tissue production. In the control group, osteoid tissue development increased with time. Rabbits tolerated allogenic rabbit teeth without rejection or antigenic response because the teeth contained fewer cells.

References

1. Billow, Damien et al. “The Reamer-Irrigator-Aspirator System: A Review.” Surgical technology international vol. 29 (2016): 287-294.
2. Goulet, Jennifer L., et al. "Deficiency of 5-lipoxygenase accelerates renal allograft rejection in mice." The Journal of Immunology 167.11 (2001): 6631-6636.
3. Greenwald, A. Seth, et al. "Bone-graft substitutes: facts, fictions, and applications." Jbjs 83.2 (2001): S98-103.
4. Baldwin, Paul, et al. "Autograft, allograft, and bone graft substitutes: clinical evidence and indications for use in the setting of orthopaedic trauma surgery." Journal of orthopaedic trauma 33.4 (2019): 203-213.

5. Pollock, Raymond, et al. "Donor site morbidity following iliac crest bone harvesting for cervical fusion: a comparison between minimally invasive and open techniques." European Spine Journal 17.6 (2008): 845-852.
6. D'Souza, Marissa, et al. "Graft materials and biologics for spinal interbody fusion." Biomedicines 7.4 (2019): 75.
7. Rasouli, Rahimeh, Ahmed Barhoum, and Hasan Uludag. "A review of nanostructured surfaces and materials for dental implants: surface coating, patterning and functionalization for improved performance." Biomaterials science 6.6 (2018): 1312-1338.
8. McINTYRE, ROBIN A. "Common nano-materials and their use in real world applications." Science progress 95.1 (2012): 1-22.
9. Priyadarsini, Subhashree, Sumit Mukherjee, and Monalisa Mishra. "Nanoparticles used in dentistry: A review." Journal of oral biology and craniofacial research 8.1 (2018): 58-67.
10. Fillingham, Y., and J. Jacobs. "Bone grafts and their substitutes." The bone & joint journal 98.1_Supple_A (2016): 6-9.
11. Fernandez de Grado, Gabriel, et al. "Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management." Journal of tissue engineering 9 (2018): 2041731418776819.
12. Wang, Wenhao, and Kelvin WK Yeung. "Bone grafts and biomaterials substitutes for bone defect repair: A review." Bioactive materials 2.4 (2017): 224-247.
13. Kim, Soo Min, et al. "The effect of copper pre-cleaning on graphene synthesis." Nanotechnology 24.36 (2013): 365602.
14. Sheikh, Zeeshan, et al. "Biodegradable materials for bone repair and tissue engineering applications." Materials 8.9 (2015): 5744-5794.
15. Fitra, Legowo, D., Utomo, B., Suroto, N. S., Parenrengi, M. A., & Al-Fauzi, A. (2021). Intracranial foreign body granuloma caused by oxidized cellulose polymer and etherified sodium carboxymethyl cellulose: an experimental study with orictolagus cuniculus rabbits. International Journal of Health & Medical Sciences, 4(2), 267-272. https://doi.org/10.31295/ijhms.v4n2.1741
16. Del Canto-Díaz A, de Elío-Oliveros J, Del Canto-Díaz M, Alobera-Gracia MA, Del Canto-Pingarrón M, Martínez-González JM. Use of autologous tooth-derived graft material in the post-extraction dental socket. Pilot study. Med Oral Patol Oral Cir Bucal. 2019;24(1):e53-e60.
17. Sohn, Hoon-Sang, and Jong-Keon Oh. "Review of bone graft and bone substitutes with an emphasis on fracture surgeries." Biomaterials research 23.1 (2019): 1-7.
18. Burdușel, Alexandra-Cristina, et al. "Inorganic Nanoparticles in Bone Healing Applications." Pharmaceutics 14.4 (2022): 770.
19. Rejab, Atalla F. "The effects of two bone substitute materials in the treatment of experimentally induced mandibular defects: An experimental study." Al-Rafidain Dental Journal 18.1 (2018): 9-20.
20. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. International Journal of Health Sciences, 5(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
21. Aroni, Maurício Andrés Tinajero, et al. "Loading deproteinized bovine bone with strontium enhances bone regeneration in rat calvarial critical size defects." Clinical oral investigations 23.4 (2019): 1605-1614.

22. Stavropoulos, Andreas, et al. "Clinical and histologic evaluation of granular Beta-tricalcium phosphate for the treatment of human intrabony periodontal defects: a report on five cases." Journal of periodontology 81.2 (2010): 325-334.