Evaluation of the Effect of Nano-Hydroxyapatite Versus Nano-Hydroxyapatite 3D Scaffold Membrane on Osseointegration's Quality of Dental Implants (Radiographic and Histopathological Experimental Study)

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Abstract

Innovation in biomaterials is always of interest in the medical field; with emergence of nano technology which enhanced biocompatibility and bioactivity of such materials are of great benefit. Nano technology treatment with biomaterials, bone graft substitutes and 3D scaffold membrane could be further utilized to enhance osseointegration, success, and survival of dental implants.

Evaluation of new bone formation around implants in rabbit's tibias supplemented by 3D scaffold membranes and nano-hydroxyapatite (nHA) radiographically and histopathologically.

A total of 24 dental implants were placed in 12 rabbit's tibiae which was assigned to Groups 1, 2 and 3. G1=implant as control group, G2=implant +bone Graft, G3=G2+membrane according to the material. Animals were euthanized after zero and 2 months. Radiographs were obtained at time of implant surgery and after 2 months. Bone quality was evaluated between the three groups radiographically, & histopathologically.

Results revealed preferences of G3 formed bone quality than G2 and G1. Radiographic results of G3 revealed best bone osteointegration over the other groups. In addition, the histopathological results revealed better osteointegration with formation of thick lamellar compact bone when using combination of nHA bone graft and 3D scaffoled membrane than other groups.

Within the limitations of this study, results demonstrated that nHA with 3D scaffold membrane has a high potential in application as an adjunctive bone substitute, improving the osteointegration and may influence bone density and quality around dental implants.

Experimental article (J Int Dent Med Res 2023; 16(4): 1414-1424)

Keywords: Nano-hydroxyapatite, 3D scaffold membrane, osseointegration, bone graft, dental implants.

Received date: 08 July 2023 **Accept date:** 02 August 2023

Introduction

With the innovation of nanostructures and biomaterials and with the goal of creating and enhancing in vivo tissue repair techniques, nanotechnology was invented as a very large subject of study that encompasses many scientific disciplines[1]. Hydroxyapatite (HA) has been the subject of investigation in the medical profession because of its biocompatible qualities and function as the main mineral in bones and teeth[2].

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Ca5 (PO4)3OH (Calcium hydroxide phosphate) is the chemical formula of hydroxyapatite (HA), which is regarded as the structural model for the mineral phase of bone, dentin, and enamel. Because of its bioactivity and biocompatibility, synthetic HA is obviously utilised extensively in medicine and dentistry [3]. In orthopaedic, maxillofacial surgery, and dental procedures, it has been utilised to fill a variety of bony abnormalities. Additionally, it might be regarded as one of the primary inorganic elements of living hard tissues. Interestingly, it is recognised as a crucial bioceramic component in artificial bone and tooth restoration. To repair a bone defect, a variety of materials can be used, including allograft, xenograft, autogenous bone transplant, and alloplast materials [4].

One of the most recent biomaterials to be used is the nano-hydroxyapatite type of hydroxyapatite (nHA). Its crystals are between 50 and 1000 nm in size[5]. It might be produced using a variety of techniques, including chemical processes and a more contemporary technology known as "template technique." This procedure is a key biomimetic synthesis process that use a template to control the crystal's development direction and produce nano-apatite with a structure and composition like that of natural bone[6].

Researchers have paid close attention to the use of nHA as a co-adjuvant material in oral surgery, particularly regarding osseointegration, osteoconductivity, and the promotion of cell proliferation[7]. According to several studies, nHA has many benefits including good bioactivity, angiogenic qualities, negligible toxicity, and the absence of inflammatory or antigenic reactions[8]. Due to their strong compositional similarity to normal bone, where bone tissue immediately binds to HA and causes the deposition of newly created bone, this proofed the regeneration capacity[9]. Clearly, it has been confirmed that HA surface supports osteoblastic cell adhesion, growth, and differentiation and can be used to restore the bone loss and functions[10]. One of the most significant biomaterials for bone reconstruction for endosseous implants in oral and maxillofacial reconstruction to date is nHA. However, there are several drawbacks to this biomaterial application that merit consideration, primarily the high cost of production. However, more advancements are needed in the performance of induced osteogenesis and the characteristics of the nanoscale materials[11]. Indeed, wide ranging studies concerned with the substitution of biological aspect by essential trace elements on trace levels have discovered that these substitutions play main roles in the biological performance[12]. For example, Na plays an important role in osteoporosis and bone metabolism, Mg has a significant role in mineral metabolism and calcified tissues. F has been well recognized for its avoidance of dental caries and enhancement of crystallization and mineralization of calcium phosphates in bone formation. Sr as trace element has been shown to have a dull effect of inhibiting osteoclast activity and stimulating osteoblast differentiation and therefore the bone formation[13]. Numerous studies accepted that the incorporation of functional trace elements can considerably improve the bioactivity of bioceramics HA. Additionally, the incorporation of Sr ions into HAP might also stimulate presence of the angiogenic factor such as vascular endothelial growth factor (VEGF) in vitro[14]. Here, great effort has been made to determine nHA effects. Thus, in this study, we aimed to identify the osteointegration effects of nHA with 3D scaffold membrane on dental implants radiographically and histopathologically in tibias of experimental rabbits.

Materials and methods

Study design

The experiment was done using twelve experimental rabbits which were divided into three equal groups according to the used material, so each leg contains different group as shown in table (1).

Rabbits	R1, R7		R2, R8		R3, R9	
Leg Side	Right side	Left side	Right side	Left side	Right side	Left side
Treatment modalities	G1	G2	G1	G3	G3	G2
Follow up (control) (CT Follow up + Pathology Follow up)						
Rabbits	R4, R10		R5, R11		R6, R12	
Leg side	Right side	Left side	Right side	Left side	Right side	Left side
Treatment modalities	G1	G2	G1	G3	G3	G2
Follow up 2 months (CT Follow up + Pathology Follow up)						

Table 1. Different groups of rabbits in the experiment: G1=implant, G2=implant +bone Graft, G3=G2+membrane.

Material	Description and composition	%
Nanohydroxyapatite (nHA)	SiO_2	0.597
	Al_2O_3	0.572
	Fe_2O_3	0.038
	MgO	1.02
	CaO	51.468
	Na_2O	1.247
	K_2O	0.053
	P_2O_5	43.57
	SO_3	0.078
	Cl	0.022
	ZnO	0.019
	BaO	0.035
	SrO	0.068
	ZrO_2	0.012
	LoI	1.20

Table 2. Description of materials used in this study.

Characterization of Nanohydroxyapatite nanoparticles

With a scanning electron microscope (SEM, Model Quanta 250 FEG, Field Emission Gun, accelerating voltage 30 KV. FEI Company, Netherlands) equipped with energy dispersive X-ray analyses (EDX), representative samples of the nHA granules were chemically examined (magnification14x up to 1000000 and resolution for Gun.1n). The average nHA crystal size and the porosities between them were measured. Composition of nHA is mentioned in table (2).

Surgical procedure and postoperative care

The experimental rabbits were prepared for surgical procedures and insertion of implants*. Twelve white New Zealand mature male rabbits aged 7-8 months with a mean weight of 2.5 kg were used in this experiment. Research Ethics Committee at Faculty of Dentistry, October 6 University, Giza, Egypt approved the study with number (RECO6U/30-2022). The animals were randomly divided into equal 3 treatment groups (4 animals/group) as presented in table (1). Before the procedures, all animals were separated from each other and then acclimatized in the laboratory environment for 5 days. These rabbits were fed by a special, pelleted commercial diet. Animals were fasted 12 hours before procedures. They all were administered general anaesthesia by intramuscular injection of ketamine hydrochloride** (50 mg/kg) 10 minutes following the premedication with xylazine hydrochloride*** (5mg/kg) muscle relaxant.

*JDental Care, Modena, Italy.
**Ketamax 50, Troikaa Pharmaceutical Ltd., India.
***Xyla-ject, ADWIA Co. S.A.E., Egypt. (For Vet. Use Only)

Surgical protocol

Once general anaesthesia had been established, the tibia region of each animal was washed with iodine after shaving. A 5-cm incision then was made along the medial aspect of the proximal tibia. Implant insertion under continuous irrigation with sterile saline, the implant installation procedure was carried out according to the manufacturer’s drilling protocol. A round head bur was used to create a saucer shape depression point in the compact bone to facilitate positioning of the spiral drills used later. The round head bur was used with exterior irrigation because it penetrates the bone only by a small degree at the implant receiving site.

The site was primarily drilled with a Diameter (D) 2.0 mm twist drill at a speed of 800 rpm, Torque 30 Ncm to the correct depth (8mm) for the fixture insertion. The initial site was prepared for all implants using a D 2.4, D 2.8mm drill. The final receptor site for D3.2 mm cylindrical fixture body was prepared with a D3.2mm drill.

Group 1 represented the control group received implants only, while Group 2 received implant with graft, finally in Group 3 the implant osteotomy was loaded with modified nHA with the 3D membrane and the implant was inserted. In G2 the nHA graft material was packed into osteotomy site followed by fixture placement, and in G3 graft was placed similar to G2 and the 3D scaffold membrane secured in place with the fixture cover screw.

Implants were gently placed into osteotomy site using fixed-torque ratchet at 30 Ncm, followed by placement of cover screw. The site of implantation was then irrigated profusely with normal saline, and the soft tissue was repositioned and approximated with black silk 1/0 suture. The area was washed with a mixture of iodine and 70% ethanol. Animals were kept in individual cages to confirm restricted mobility immediately postoperatively. After surgery, each animal received intramuscular Ceftriaxone* antibiotic at a dose of 250 mg, 50 mg / kg once daily 3-5 days and Voltaren** analgesic at 5 mg/kg for 3 successive days starting second day postoperative, Figure 1 (a-f).

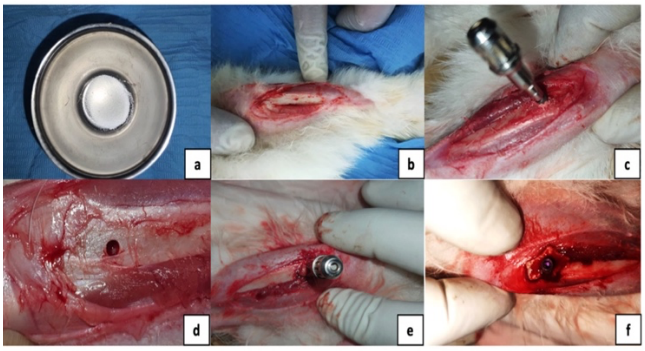

Figure 1. Photographs showing steps of surgical procedures and insertion of implants with nHA membrane. a: nHA particles, b: Shaving and preparation of insertion area in Tibiae, c: Insertion of the drill, d: Opening of the implant’s hole, e: Insertion of implants, f: Implant’s placement.

*Ceftriaxone 500 mg vial, manufactured by SANDOZ a Novartis division, Egypt.
*Diclofenac natrium 75mg/3ml ampule, manufactured by Novartis, Egypt.

Preparation of slides for Histopathological examination

The bones of the tibiae were immediately fixed in 10% formalin following the experimental animals' death, and they were subsequently decalcified in EDTA for 4 weeks. Then were put through standard histological processes to create tissue sections with paraffin embedding that were 5 mm thick. The acquired sections were stained with hematoxylin and eosin (H & E) stain for histopathological inspection so as to assure the proper histopathological evaluation. In order to identify bone trabeculae, the slides were also dyed with Masson trichrome.

Radiographic Assessment

The radiographic assessment was done using Multi-slice CT scan (Toshiba Alexion 16 Slice CT Scanner Machine) which was operated on living rabbits at base line, and two months after implant placement for bone density assessment. A slice thickness of 0.5mm, Recon interval 0.1, image filter UO2, with exposure parameters of 100KV and 80 MAS was used. Statistical assessment of the collected results was formed.

Statistical analysis

Numerical data was represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Data showed parametric distribution and were analysed using two-way ANOVA. Comparisons of simple main effects were done through utilizing the error term of the two-way model with p-values adjustment using Bonferroni correction. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.2.3 for Windows[1].

Results

Characterization of nHA

The characterization results of nanohydroxyapatite crystals revealed natural texture, which was significantly affected by the low crystallinity, small crystal size (50 nm in length, 2.5 nm in width and 2.5 nm in thickness) and ion substitution, (Figure 2: A).

Clinical and physical examinations

Six of the experimental rabbits remained healthy during the whole experiment and recovered quickly after surgery. There were no symptoms of adverse effects or infections in these rabbits.. Three rabbits died after the opearation due to presence of infection in site of surgery.

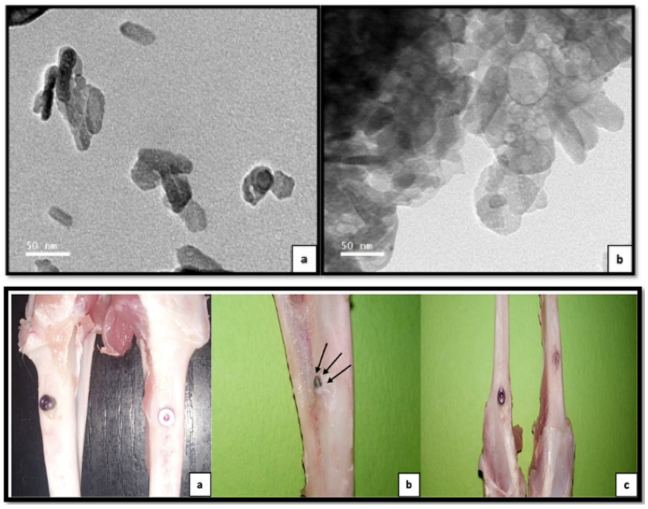

Figure 2. A: Photomicrographs of characterization of nHA using SEM.
B: Photograph showing macroanatomy after euthanization, a: both tibias with implants, b: G2, c: right leg showing complete healing with G3.

Macroanatomy evaluation

The macroanatomy of the rabbits was checked after euthanization and before the declacification step. In G2 group, one rabbit showed soft tissue infection ended by fistulae**.** The healing of G3 showed better clinical results than G1 and G2, (Figure 2: B).

Histopathological evaluation & Histochemical findings

Control

The H& E slides were examined for detection of osteointegration of bone around the implants. Regarding the control group **(G1),** the bony defects around the implants were clearly observed. After two months, the connective tissue stroma filled the defects which showed granulation tissue formed with blood vessels noticed and slight areas of bone trabeculae started to form as normal healing which revealed irrelevant bone osteointegration around the implants. In most of cases, the periphery of the bony defects around implants were mainly formed of thin trabeculae of bone, figure 3 (a-c).

Nanohydroxyapatite only (G2)

Regarding G2, the group's slides revealed good results through the whole experiment period. Firstly, At the margin of the implant sockets, regions of woven bone were frequently observed, encircled by swollen connective tissue cells. After two months, lacunas with osteocytes as a sign of healthy bone, were seen. In this group, the implant's surface stimulated an inflammatory response in some rabbits. In addition, new bone formation around the implants were noticed as woven bone with slightly basophilic bone with many osteocytes haphazardly arranged. Furthermore, the examined sections showed some reactive new bone formation around the implants in rabbits. There was some periosteal bone formation at the cortical drill hole site, globular appearance of the newly formed bone with numerous large osteocytes was obvious (Figure 3: d-f).

Nanohydroxyapatite + membrane (G3)

In the G3 group, the histopathological findings exhibited better osteointegration in most of the cases than those of G1& G2 groups. Regarding the control, a cartilaginous tissue in two rabbits was a consistent feature and the rest showed starting of bone formation. The surrounding of the implant was filled with a dense network of lamellar bone trabeculae in all the studied rabbits after two months. Bony spicules contain moderate number of osteocytes and few amount of bone marrow within the space. Active remodeling with numerous reversal lines were revealed symbolizing of the newly formed bone, (Figure 3: g-i).

Histochemical findings

The slides were examined after staining with Masson trichrome after two months. In G1, the lining was with minimum bone formed. As opposed to that, the network of bone trabeculae in G2 showed bone with many areas of bone marrow. Additionally, G3 demonstrated well-calcified compact bone with sparse regions of bone marrow that extended from the usual bony edge (figure 3: B (a-c).

Radiographic evaluation

Multi-slice CT scan was operated on living rabbits after implant's placement for bone density assessment. The radiographic pictures revealed different bone osteointegration density throughout the pre-planned study design time. Obviously, G3 results revealed enhanced bone formation surrounding implants than other groups. Statistical analysis was performed with R statistical analysis software version 4.2.3 for Window, (Figure 4: A).

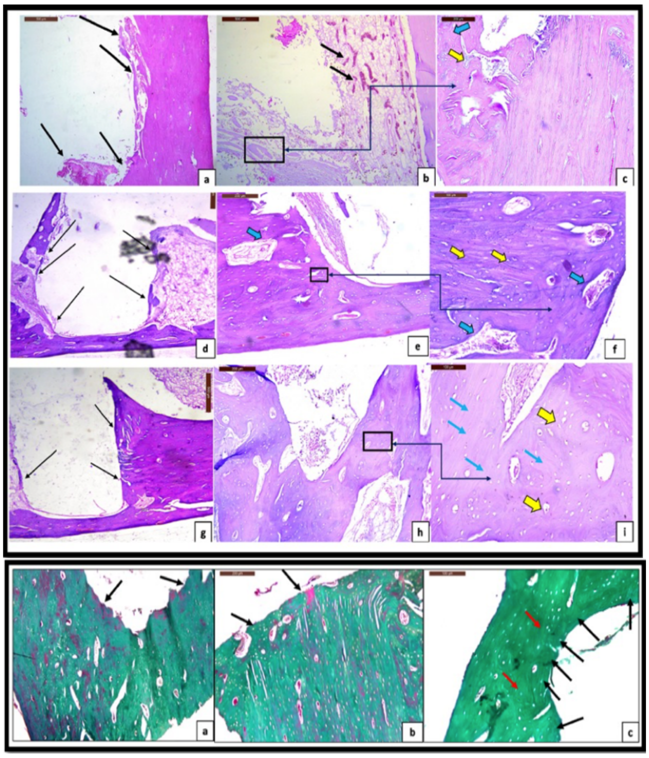

Figure 3. A: Photomicrographs showing G1, a: Control showing some empty areas around implants (H& E x40), b: After 2 months, the specimens showed granulation tissue formed with blood vessels noticed (black arrows). Areas of bone trabeculae (black box) started to form, (H& E x40), c: Higher magnification showing bone marrow (yellow arrow) and new areas of bone surrounded the implants, (blue arrow), (H& E x 200). Photomicrographs of G2, d: Control showing signs of new bone formation were evident in some areas in the tissue surrounding the implant (black arrows), (H& E x 40). e: after 2 months, some areas revealed formation of woven bone around implants with considerable amount of bone was observed extending from the endosteum and surrounding the implant, bone marrow spaces were noticed, (H& E x 100). f: Higher magnification, revealed bone marrow (blue arrows), The newly formed bone was characterized by globular appearance with numerous large osteocytes (yellow arrows), (H& E x200). Photomicrographs showing G3, g: Control showing the socket surrounding the implant with little bone formation, (arrows), (H& Ex 40). h: after 2 months, lining of socket showing formation of compact bone (H& E x100). i: Higher magnification showing the formed bone was completely remodeled to mature lamellar bone with flattened osteocytes (arrows), reversal lines (blue arrows) (H& E x 200).

B: Photomicrographs showing the three groups with (Masson Trichrome stain), a: G1: lining of implant interface showing formation of little bone, (black arrows), (H& Ex100). b: G2: formation of woven bone in implant interface, (black arrows), (H& Ex100) c: G3: lining with formed compact bone (black arrows), resting line (red arrows) (H& E x100).

Group / Time	Density (HU) (Mean±SD)			f-value	p-value
	G1	G2	G3		
Zero days	191.44±35.52^{B}	454.88±35.12^{A}	481.41±101.22^{A}	9.11	0.002*
2 months	1105.06±43.13^{C}	1820.50±107.50^{B}	2464.75±203.84^{A}	163.98	<0.001*
f-value	147.94	330.50	697.19		
p-value	<0.001*	<0.001*	<0.001*		

Table 3. Comparisons of different materials effects.
Values with different superscript letters within the same horizontal row are significantly different *significant (p<0.05).

Statistical Results

Results of materials' effects comparisons presented in table (3), which showed a significant difference at zero day between tested groups (p<0.001), with G2 and G3 having significantly higher density values than group (1) (p<0.001). After 2 months, the difference was also statistically significant (p<0.001), but with all pairwise comparisons being statistically significant (p<0.001) and with G3 having the highest density, followed by G2, then G1. For all groups, there was a significant increase of density after 2 months (p<0.001).
The mean bone density in newly formed bone area was 191.44,454.88, 481.41.for the G1, G2 and G3 groups in order at base line respectively, while after 2 months, the results was 1105,1820.5,2464 for the G1, G2 and G3 groups respectively. Changes in mean bone density in area of bone formation surrounding implants in different groups showed a statistically significant difference in bone density values in G3 group, followed by G2 then G1both at base line with p value (0.002) and after 2 months with p value (<0.001). Mean and standard deviation values for density are presented in figures (4: B1) and (4: B2).

Discussion

The study of bone tissue engineering has paid a lot of attention to nanotechnologies, which are connected to a wide range of academic fields including physics, chemistry, engineering, the life sciences, and medicine. No single biomaterial is optimum for every craniomaxillofacial application. Despite having many benefits, conventional tissue replacement techniques (such autografts and allografts) also have a number of drawbacks that must be addressed for each patient[15]. Nanotechnology was developed as a result of Feynman's ambition to develop new technologies and materials that are better and more effective[16]. The fundamental principle behind nanotechnology is the use of single atoms and molecules to create useful structures. The nanoparticles have increased availability in biological systems because of their nanoscale size. Rapid development in the field of biomaterials nanotechnologies helps to give a remedy for the growing need for effective bone grafts and implants[8].

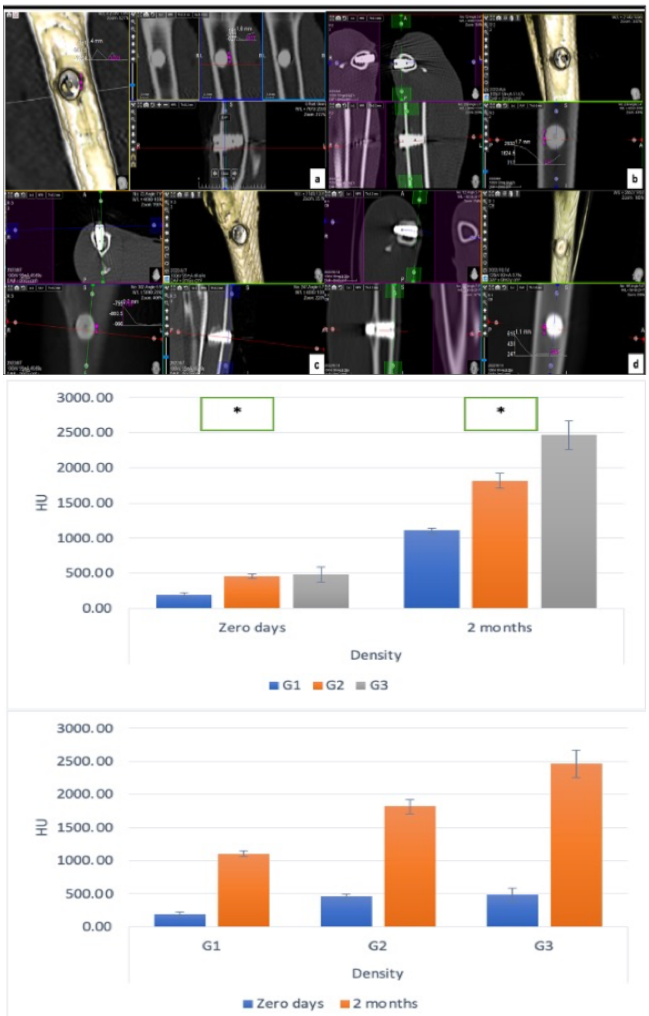

Figure 4. (A): Radiographic pictures of different groups, a: G1 after 2 months, b: G2 after 2 months, c: G3: control, d: G3:2 months.

(B1): Bar chart showing mean and standard deviation values for bone density (A).
(B2): Bar chart showing mean and standard deviation values for bone density (B).

According to current definitions, nanomaterials are substances with fundamental structural units smaller than 100 nm in at least one dimension. They also have special qualities that set them apart from their bulkier counterparts and have helped them become very successful in a variety of biomedical applications. Superior physicochemical qualities result from a material's drastic rise in surface area, roughness, and the ratio of surface area to volume at the nanoscale. Cell signalling, cell growth, cell viability, and cell integration are all impacted by these nanoparticles. moreover, nanoparticles influence osteoblast activity, proliferation, differentiation, and migration in addition providing cellular support[17].

Osseointegration, a complicated process, is essential for the clinical effectiveness of bone-anchored dental and orthopaedic implants. A substance that is biocompatible, osteoconductive, osteoinductive, and accepted by the patient's immune system is necessary for bone regeneration. Indeed, bone repair is a difficult process involving cellular activity, mineralization, and remodelling of the damaged area to restore the original structure[18]. Therefore, there have been numerous studies conducted to improve these intricate procedures. According to recent research, three-dimensional pore structures may help with fibrovascular and nerve colonisation as well as cell adhesion, differentiation, and proliferation, allowing the new bone to "crawl" into the material and lead to early osteoinduction [19].

The choice of nHA powder was justified by its frequent application in bone regeneration techniques, such as pre-prosthetic surgery to thicken the alveolar ridge[20]. Nanohydroxyapatite (nHA) is the major inorganic component of bone exhibiting hexagonal crystal structure, biocompatibility, osteoconductivity, higher stability, and larger surface area with osteointegration property. As previously mentioned, Nano-hydroxyapatite possesses clear influence on early bone formation. Additionally, the nanostructure of this synthetic bone substitute materials, is closely resemble the nanostructure and texture of the natural HA of bone with its subsequent enhanced cellular activity and bone induction potential[21]. Due to its delayed resorption, HA after implantation in the body may continue to be integrated into the regenerated bone. It is frequently added to polymers in the form of nanoparticles (nHA) to enhance their mechanical characteristics and to coat metal implants to increase their bioactivity. The main objective is to mineralize the organic matrix with calcium phosphate, notably with HA nanoparticles, which are the most stable and resemble in vivo mineral phase. In addition, natural bone apatite and nHAs share many physico-chemical and biological characteristics[22]. In addition, trace elements such as Strontium and Magnesium play an important role by replacing calcium ions and forming phosphate compounds that are capable of increased cellular proliferation[23] So, application of these materials experimentally gives the chance to study the properties widely.

The New Zealand rabbit is reportedly one of the most often used animal species in medical research. Compared to rats and mice, rabbits are easier to handle, hence they were chosen for the current study. Additionally, rabbits have their systems, which is a substantial benefit in terms of the validity of the results since tiny rodents lack these systems and thses rodents have defined Haversian systems that are not identical to those in humans[24]. The ease of handling and the appropriate size with the minimal stated differences between human and rabbit bone composition and density facilitate their wide use in musculoskeletal research studies. Rabbit was chosen for this research for several reasons. It offers the benefit that, in comparison to rats, mice, or guinea pigs, greater bone abnormalities can form more quickly. Additionally, by using only males, the potential hormonal changes that may occur in females were avoided, as this variable would have compromised the reliability of the findings. When compared to other species like some rodents and primates, the rabbits also exhibit faster skeletal alterations and bone turnover. To reduce the growing effect, adult animals must be used in the establishment of an experimental model for the study of animal craniofacial abnormalities. As a result, the adult New Zealand rabbits were used which were about 7-8 months old (adult age). The tibia of rabbits was chosen because their anatomical region is characterized by low mechanical stresses and relatively stable neighboring components. In order to explore the interactions between newly generated bone and in situ bone, a protected environment has been created[25].

Most of the current bone regeneration techniques show acceptable results, but only in relative terms. Likewise, their use and accessibility have disadvantages and restrictions. Furthermore, their effectiveness and cost-effectiveness have been questioned by a number of contentious reports. There are currently no synthetic or heterologous bone substitutes that are superior to, or possibly even identical to, bone in terms of their biological or mechanical qualities. The permanency period and material absorption at the site of the bone defect determine how well the repair will work[26].

Nanohydroxyapatite particles revealing unique characteristics. The analysis of nanohydroxyapatite particles (nHA) in the present investigation revealed that the shape of nHA consisted of rod- or nanoneedle-like particles with an average particle size that was observed to be between 20 and 50 nm. The same results were obtained in a subsequent study[27].

Considering the macroanatomy examination, the present study revealed good results of healing around implant's site. During progressing in experiment time, a part of the bone appeared on the surface of the implant which indicated improvement in contact between the implant and bone with time resulting in better mechanical properties of bone implant interface. This was agreed with Gehrke et al [28]and Pang et a results.[29]

A radiographic exam is required. Additionally, the "gold standard" for assessing bone development has been referred to as histological analysis. This approach does have certain limitations, though, namely the possibility of harm to the interface and results when cutting and grinding the implant into bone blocks. Furthermore, two-dimensional histology may not have captured the true condition because of its restricted capacity for assessment because it may depend on the location from which the section was collected[30]. That's why radiographic evaluation was a main goal in this study evaluation for newly formed bone in different groups. Furthermore, the deposited bone matrix was characterized by using Masson trichrome as a proximate indicator of the amount of newly formed bone[31].

The radiographic results of the present study revealed different bone density values throughout the different groups. The statistical results revealed significant difference in group G3 over both G1& G2 at zero and 2 months, revealed that mixing of membrane and graft enhance bone formation. These outcomes may be correlated with some research using hydroxyapatite nano gel which enhanced osteogenesis, and lead to improvement in bone healing as nanohydroxyapatites which has a nanostructured surface with higher surface area and higher reactivity, letting them to bind to bone creating a biomimetic coating on implants[32].

A recent study by Mohammed et al.,[33] discussed the radiological effect of nanohydroxyapatite gel on defects in tibias of dogs. They revealed that this material accelerated the healing process. Their results were in accordance with the present study results, and they clarified that using of a xenograft supported with hydroxyapatite nano gel has been advocated with success. This is evident from the results of the radiological examination in the present study which showed significant difference in G3 group, which agreed with previous study[34]. They concluded that this material can be considered as one of the most promising materials for its highly biocompatible and osteoconductive scaffolds to be used in bone tissue regeneration.

The results of the current investigation showed that G3 outperformed the other groups in terms of its effects. The histological findings of Ibrahim & Al-Ghaban.,[35], who proved that the thread sites surrounding implants were filled with new bone with osteocyte cells and osteoblast lining the harversian canal after 6 weeks, with fibrous connective tissue and new blood vessels and a delay in bone formation, nearly matched our findings. This was explained by the fact that coated implants placed in living bone osseointegrated under the normal biological conditions for bone growth. They also mentioned that with time, the association between osseointegrated implants and bone formation became more rigid.

Another study by Zaki et al.,[36] revealed a point of view which is in accordance with the present study results. They pointed out that placement of nHA bone graft showed very good results in this study through its great accessibility for bone formation. They also noted that nHA has outstanding stiffness, hardness, dimensional stability, and biocompatibility. Since nHA is similar in size to natural inorganic minerals found in bone and shares the same shape, structure, and composition as apatite in human bone, it is easier for cells and molecules in the human body to recognize it.

One further study by Hassan & El-Haddad, [37] pointed out good results considering nHA. Their findings, which demonstrated the growth of lamellar bone and the production of primary osteon, were in agreement with the findings of the current experiment. They added that an increased in bone area was measured which was more apparently in nHA treated rabbits and was explained that a positive effect of HA on the alkaline phosphatase activity in osteoblast cells occurred. However, Renzo et al., [38], results indicating that dental implants placed in sockets preserved with various biomaterials, present good clinical performance similar to implants placed into native bone.

Karazisis et al.[39] used an experimental animal model to study the effects of nanohydroxyapatite alterations on implant surfaces. They found that these modifications reduce the inflow of preimplant macrophages and the early expression of inflammatory and osteoclastic genes. Additionally, their findings demonstrated increased osteogenic activity at the Nanoimplant.

Besides, nHA has good osteoconduction properties, and good mechanical properties, like those of human cortical bone. nHA has better biological performance, and composite materials synthesized from nHA are becoming the first choice of replacement material for treatment of bone defects[40].

Conclusions

Research on bone tissue engineering currently places a high priority on nanomaterials. However, bone can heal and change on its own. In many instances, external convention is necessary because these mechanisms are insufficient. To be able to replace bone and encourage regeneration, biocompatible materials are required. Nanomaterials, such Nanohydroxyapatite, showed remarkable potential in osteointegration and appear to be particularly promising for these applications. In addition, Nanohydroxyapatite with 3D scaffold membrane enhanced osteointegration results than nanohydroxyapatite alone due to enhancement of the chemical properties that motivate osteointegration.

List of abbreviations

nHA: Nanohydroxyapatite
HA: Hydroxy Apatite
nm: nanometre
Ca5 (PO4)3OH: Calcium hydroxide phosphate
VEGF: vascular endothelial growth factor
Na: Sodium
Mg: Magnesium
R: Rabbit
mm: millimeter
EDX: energy dispersive X-ray
H& E: Hematoxylin and Eosin
SD: Standard Deviation
cm: centimetre
SEM: Scanning Electron Microscope
G: Group
Sio2: Silicon Dioxide
Al_2O_3: Aluminium Dioxide
Fe_2O_3: Iron (III) oxide or ferric oxide
MgO: Magnesium oxide
CaO: Calcium Oxide
Na2O: Sodium Oxide
K2O: Potassium oxide
P_2O_5: Phosphorus pentoxide
SO_3: Sulfur trioxide
Cl: chloride
ZnO: Zinc oxide
BaO: Barium oxide
ZrO2: Zirconium dioxide

Acknowledgements

All authors had substantial contributions in the conception and design of the current study, where H.F & W.E, S.E: performed the surgical part of the experiment. S.F was responsible for the interpretation and performing the radiographical part. Y.A: Performance of the histopathological part. H.Y: revising the whole manuscript. All authors were responsible for drafting and revising the manuscript and approved the manuscript submission in its current form.

Limitation of the study

Although many insights were evaluated by the study, it still had limitations. Further studies will be useful for showing results in longer time duration more than two months to be able to evaluate osteointegration effects through wide range of time. Moreover, combining nanohydroxyapatite with other biomaterials can be also considered for research with other alternatives to be used.

Ethical committee

The study was approved by Research Ethics Committee at Faculty of Dentistry, October 6 University, Giza, Egypt, with approval number: RECO6U/30-2022.

Declaration of Interest

The authors report no fund was granted for this study, no conflict of interest.

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