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Comparing nano-hydroxyapatite to Deprotenized bovine bone through a two-stage maxillary sinus floor elevation

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Abstract
The purpose of this study is to compare the use of Nano HA (Nanostreams) as a predictable augmentation material for two-stage maxillary sinus floor augmentation compared to deprotenized bovine bone (Tutogen) Twenty patients from both sexes between the ages of 34 –52 years were enrolled in this study. Patients were all indicated for lateral window sinus lifting with residual bone height of less than 5 mm. A titanium micro mesh was placed into the sinus and fixed to the lateral wall to maintain the elevated Schneiderian membrane in place. The twenty patients were divided into two groups, Nano hydroxyapatite (Nano streams) was used as the grafting material in the first group (Test group) while in the second group, a deprotenized bovine bone (Tutogen) was used as a control grafting material. An absorbable collagen membrane was used to cover the lateral window. An immediate and Six-month postoperative CBCT were carried out for all patients and the height of the newly formed bone beneath the mesh measured when the implant was inserted, bone-core biopsies were obtained by means of a trephine bur and submitted for histomorphometric analysis. a typical residue vertical ridge height among the 20 sinuses preoperatively was 3.68 mm +/- 0.95 mm in the Nano HA group and 3.74 mm +/-0.59 mm in DBB group. The vertical ridge height as measured on the 6-month postoperative CBCT average 12.1 mm +/- 1.53 mm for the Nano HA group and 12.6 mm +/-1.11 mm in the DBB group. The average new bone percentage in the Nano HA group was 29.84% -/+ 6.7% and 34.73% -/+ 7.9% in the DBB group. Statistical analysis proved there was no discernible difference between the two groups in terms bone height gain and percentage of new bone formation.

Keywords: Nano hydroxyapatite, Deprotenized bovine bone, histomorphometric

1. Introduction
Due to the widespread usage of dental implants to replace lost teeth, difficult surgical methods have been developed to enhance the amount of bone that is readily available in order to provide primary stability. Due to an inadequate posterior alveolar ridge, poor bone quality, and increased maxillary sinus pneumatization, placing dental implants in an edentulous posterior maxilla may be challenging.

To address the bone deficit in the edentulous posterior maxilla, a number of procedures have been devised, including sinus lift techniques (open-closed), short implants, tilted implants, and onlay bone. Grafts [1].

Since the introduction of sinus floor elevation by Tatum in 19772 and Boyne and James in 1980 [3], researchers have been evaluating bone graft materials to determine those best suited for this procedure. In implant dentistry, autologous bone grafts continue to be regarded as the gold standard for bone regeneration. Yet, because there is a limited supply of bone in the mouth cavity, bone must frequently be taken from an extraoral region. Also, taking into consideration the higher morbidity & patient discomfort during autogenous bone harvest4, a variety of alloplastic, xenoplastic, and allograft materials are suggested to substitute autogenous bone. Most of these bone substitutes show only osteoconductive potential and serve as a framework for bone growth [5, 6].

One of these bone substitutes is hydroxyapatite in a Nano size less than 50 nms which serves as a framework for bone growth with good results but yet under investigation [7].

In the current study Nano hydroxyapatite is used for maxillary sinus floor augmentation in order to evaluate its efficiency when compared to the most commonly used xenograft bovine bone.

Aim of the study

The purpose of the current study was to quantify the increase in bone height and quality of bone remodeling following maxillary sinus floor augmentation using Nano hydroxyapatite (Nano streams) versus Deprotenized bovine bone (Tutogen).

Material and Methods

This study involved 20 patients who sought implant rehabilitation for their posterior maxillary teeth and were chosen from the outpatient clinic of the faculty of dentistry at Cairo University's department of Oral and maxillofacial surgery.

Twenty sinuses in 20 participants were enrolled and randomly allocated equally to control and test groups using sequentially numbered, sealed envelopes.

Group A: Patients underwent sinus floor elevating and enhancement using Nano hydroxy-apatite bone (NHA)1 (Test group)

Group B: Patients underwent maxillary sinus membrane elevation and augmentation using

Tutogen bovine bone (DBB) 2 **(control group)**

I. Pre-operative preparation

1) Medical evaluation

Each patient was interviewed in order to obtain a comprehensive, history, including full medical and dental history, the collected data were documented in a standard sheet. (Appendix A).

2) Pre-operative Clinical evaluation

Comprehensive detailed intraoral examination was performed including:

1. With the face bow registration, mounting on a semi-adjustable articulator, and diagnostic wax-up, a 1ry impression is taken to create a diagnostic cast.
2. Periodontal condition (Gingival index) [69].
3. If the teeth need to be restored pre surgically
4. Interarch space (To assess the need for vertical augmentation)
5. Soft tissue biotype: Thin or thick (To assess the need for soft tissue grafting 1 Hydroxyapatite nanoparticles ([NHA; Nano-streams, Cairo, Egypt] Email nanostreams@gmail.com)
6. Tutogen bone (Tutogen Medical GmbH, Neunkirchen am Brand, Germany

3. Radiographic records

a**) Plain panoramic radiograph**

• Preoperative panoramic radiograph was ordered as a primary assessment for each patient to gather information on the height of the remnant alveolar bone and to look for any remanent roots or localized bony pathosis. (Figure 2)

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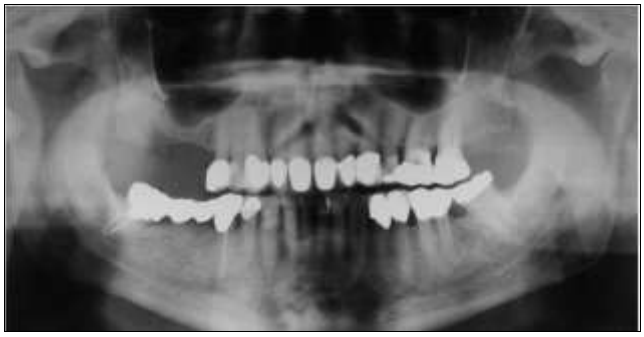

Fig 2: Screening preoperative panoramic radiograph for assessment of the intended operative site (the upper right posterior region).

b) CBCT, or cone beam computed tomography

• A cone beam computed tomography (CBCT) was ordered to exactly measure the amount of remaining alveolar bone dimensions

2. **Operative procedures**

Two steps of operational procedures were carried out

• **First stage surgery**: maxillary elevation of the sinus floor via a lateral window method according to Boyne and James 3 the created volume after elevation was maintained with a titanium mesh and grafted with either Nano hydroxyapatite bone particles or Deprotenized bovine bone.

• **Second stage surgery:** Core biopsy samples were taken from the intended implant sites at the time of implant insertion.

The use of local anaesthetic (40 mg of articaine hydrochloride combined with epinephrine (1: 100,000) for all surgical procedures [3].

First stage surgery

Preoperative Medication

All patients received prophylactic antibiotic: 2 g of amoxicillin4 (Or clindamycin 600 mg5 if penicillin allergy presented) orally 1 hour before surgery. Patients washed with (0.1%) chlorhexidine mouthwash before surgery for 1 min.6

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Surgical procedure

1. Three lines mucoperiosteal pyramidal flap was started with sufficient mesially and distally along the crestal incision to enable flap reflection., proper exposure of the region of interest and to guarantee that the flap is properly closed over a completely intact bone (Figure)
2. After the flap's reflection, a sinus floor elevation According to Boyne and James 1980, a lateral strategy was used [3].
3. Beneath a cold (4 to 5 °C) saline irrigation Using a No. 8 diamond bur was used, a rectangular bony window with rounded edges “trap door” on the lateral sinus wall. and carefully in-fractured Then, after separating the sinus mucosa, rotated medially in the direction of the sinus (Trap-door technique).
4. Millimetres thickness dynamic micro titanium mesh 7 was fixed to the lateral sinus wall by titanium screws70 to preserve the created space and the elevated schneiderian membrane. A foil template was used to adjust the dimensions of the titanium mesh before fixation.
5. The area created by raising the sinus membrane was grafted either in the form of a synthetic particulate of Nano Hydroxy-Apetite in the test group [Figure 7] or with deprotenized bovine bone (DBB); in the control group [Figure 8].
6. An absorbable collagen membrane is used following bone transplantation [8] was placed over the lateral window and extended at least 3 mm past the prepared window's perimeter to prevent soft tissue invasion and stabilized using sutures in a cross pattern (criss-cross suture) [Figure 9]. Primary wound closure was then achieved using prolyne 3/0 suture material [9].

Postoperative care

1. Each patient and his escorts received post-operative instructions that included using cold packs for 10 minutes every 30 minutes for 24 hours and following stringent dental hygiene practices including using mouthwash on a regular basis use of toothbrush and antiseptic mouthwash.
2. For the first 24 hours following surgery, patients were urged to refrain from applying any positive or negative pressure to the nasal canal (Such as blowing their noses, sipping via a straw, spitting, and breathing downward).

The postoperative medication regimen included

1. Sulbactam/Ampicillin 1 gm tablets10 is an oral antibiotic (every 12 hours for 7 days)
2. or Clindamycin pills, 300 mg11 (every six hours for 10 days if allergic to penicillin).
3. Antiinflammatory analgesics Ketorolac tromethamine For the first 24 hours, administer 30 mg/amp/2 ml via i.m. injection every 12 hours.
4. Methylprednisolone Acetate 80 mg/ml I.M. single dose for postoperative inflammation13.
5. Triprolidine HCL 2.5 mg tablets + Pseudoephedrine HCL 60 mg as systemic decongestants (every eight hours for seven days)14
6. Nasal decongestant: Oxymetazoline HCL 0.25% nasal drops15 (3 times every day for 1 week.
7. Chlorhexidine Gluconate O.1% mouthwash 16 (3 times daily for a couple of weeks)

Clinical evaluation: Postoperative follow-up and assessment

Following first-stage surgery, patients underwent routine clinical evaluations at the following intervals: 48 hours, 1 week, 2 weeks, 1 month, and then monthly until 6 months postoperative.

We looked for any evidence of bleeding, hematoma, infection, wound dehiscence, or mesh exposure in the intraoral wounds. For any indications of sinusitis, patients were questioned and given physical examinations.

Radiographic assessment

1. A CBCT scan was performed immediately after surgery and six months later to assess the development of new bone (Figure 10).
2. At four reference points that corresponded to the centres of four mesh holes almost in the potential implant sites, the distance between the crest of the ridge and the floor of the sinus was measured (Figure 11). The mean of the four measurements was then calculated from the 6-month CBCT from the crest of the ridge to the newly formed floor using measurements from the reformatted panoramic and cross-sectional views.

3. Second stage surgery

4. Operative procedure

A three-line muccoperosteial pyramidal flap [identical to first-stage surgery but with shorter oblique incisions] was reflected after a six-month healing time.

Bone core biopsy samples were obtained during implant insertion using a 3-mm-diameter trephine, under sterile saline irrigation at a cool temperature of 4 to 5 °C, and then processed for histologic and histomorphometrical examination. The samples came from the same location as the intended implant implantation.

Sequential drilling continued until the proper drill size for proper implant placement was reached, and then implants were positioned [figure 13] in accordance with the preoperative plan, which was directed by the CBCT.

The flap was finally modified and stitched.

1. All patients received postoperative instructions and medicine prescriptions:
2. Oral antibiotics: Clindamycin 150 mg capsules18 or Ampicillin/sulbactam 1 gm tablets17 (every 12 hours for 7 days) (three times daily for 10 days if allergic to penicillin).

3. Analgesics that reduce inflammation Every two times per day for the first 2 days, provide an I.M. injection of ketorolac tromethamine, 30 mg/amp/2 ml [19].
4. Mouthwash containing 0.1% chlorhexidine gluconate (Every 8 hours for two weeks).
5. A standard panoramic radiograph was taken immediately following surgery to confirm that the implants were in the right place.

Histological assessment

All core biopsies were immediately inserted into a glass container filled with 10%, PH 7.7 formalin and fixed for one week, subsequently decalcified and treated using a mixture of formic acid and ethylene-diamine-tetra-acetic acid (EDTA). Specimens were then oriented uniformly and longitudinally implanted into paraffin blocks to mark and distinguish the newly produced bone end from the native bone end.

Further, using a microtome, 5-mm-thick slices perpendicular to the longitudinal axis were created and processed with the common procedures for Masson's trichrome and hematoxylin-eosin (H-E) stainings.

The specimens were evaluated for the type of bone, the presence of osteoblasts, osteoclasts, residual graft particles, fibrosis, vasculature, and indications of the invasion of mononuclear cells or mixed inflammatory cells.

Histomorphometric analysis

A camera and computer were connected to an Olympus CX20 microscope for use in examining all of the stained sections. Images of the slides were obtained and saved as figure files, and an image analyzer computer system performed image analysis on each of the native and newly created bone's five most representative fields per specimen using the photos were opened on the computer screen using the Image J software (NIH, version 1.45e, USA).

Statistical analysis

SPSS (Statistical program for the Social Sciences), version 15, from Echosoft Inc. in the United States was used for the statistical analysis. Data were shown as mean plus/minus standard deviation. To compare every pair of the investigated variables within the investigated set of patients, a paired sample student t-test was performed. In order for a test result to be deemed statistically significant, the P-value has to be equal to or lower than 0.05.

Results:

Clinical Results

There were 20 patients in the trial, with an age range of 34 to 51, including 9 men and 11 women. for the test group (Nano HA) with average 40.2±4.9 and age range 34 to 52 for the control group (DBB) with average 44.5±6.9 with no statistically significance among the 2 groups. [Table 1].

First stage surgery

1. No intraoperative complication were encountered except for patients No 3 (control) & No12 (test), where the elevation caused a rupture of the sinus membrane. The complication was managed by using a collagen membrane to seal the hole away from the bone graft particles [Figure 15]
2. Early postoperative follow-up was uneventful and did not involve any issues with infection or dehiscence, bleeding or hematoma. Patients presented after 1 week with minimal oedema and tenderness. There were no reported signs or symptoms indicating sinusitis.

The rest of follow-up till the second surgery went without noticable complications.

Table 1: The patient's demographic data

Gender	Age	Patient No.
M	35	1
M	34	2
F	36	4
F	44	9
M	39	10
F	42	12
F	39	13
M	42	14
F	51	15
F	40	17
	40.2 ± 4.9	Mean

Gender	Age	Patient No.
F	52	3
M	34	5
F	50	6
F	47	7
M	46	8
M	36	11
F	37	16
F	53	18
M	48	19
M	42	20
	44.5 ± 6.9	Mean

Test group (Nano HA) Control Group (DBB)
F= Female / M= Male

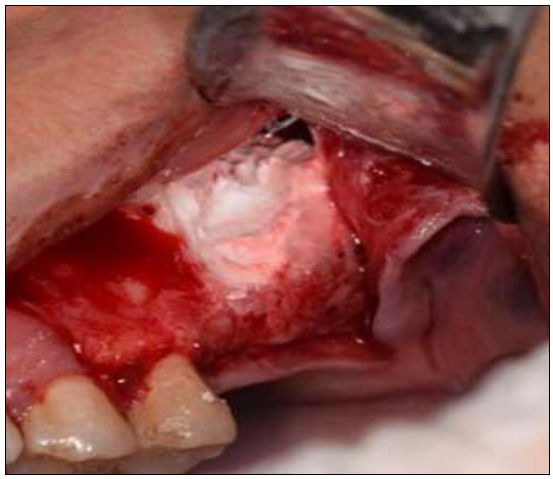

Fig 3: Grafting the sinus with Nano HA graft particles

Second stage surgery

The core biopsies were taken out for histomorphometric assessment and the endosseous implants were put in place with satisfactory initial stability at the core sites. A total of 26 implants [21] were placed in the twenty sinus floor elevated sites. The early postoperative follow-up went uneventful. The titanium mesh showed no signs of infection with complete bone integration around the titanium mesh seen in some cases [figure 16].

Radiographic Results

Immediate post-operative CBCT showed radio-opacity of the bone graft particles below the titanium mesh [Figure 17]. The (CBCT) six months after surgery revealed fresh bone development beneath the mesh. In cross sectional images

mounted to the lateral wall of the sinus, the meshes were visible. About all of the investigated wounds showed a line of demarcation separating the newly produced bone from the native bone. No signs of infection or connective tissue proliferation into the sinus cavity

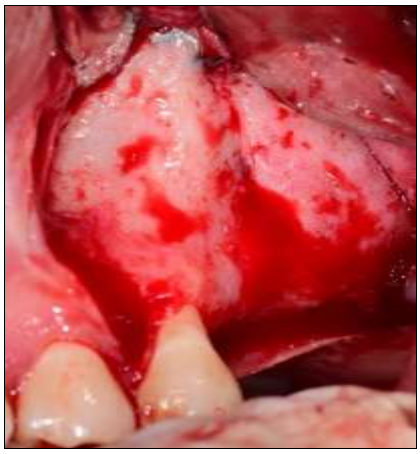

Fig 16: Complete osseintegration between the titanium mesh and bone with no signs of infection or inflammation

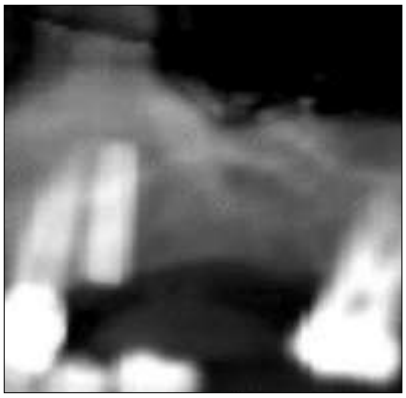

Fig 21: Reformatted panoramic view for case no 14 (Nano HA group

Bone height

In the test group (NHA), preoperatively, the original bone height varied from 2 mm to 4.9 mm (mean 3.68 +/- 0.95 mm). The height of the bones varied from 10.3 to 14.3 mm (Mean 12.4 +/- 1.58 mm) immediately following surgery, and varied between 10.2 and 13.9 mm (mean 12.1 +/- 1.53 mm) at six months (Table 2).

In the control group (Xenograft), Prior to surgery, the original bone height varied from 2.8 mm to 4.7 mm (mean 3.74 +/- 0.59 mm). Immediately following surgery, the bone height ranged from 11 mm to 14 mm (mean 12.8 +/-1.13 mm), and six months later, it ranged from 10.8 mm to 13.7 mm (mean 12.6 +/-1.11 mm) (Table 3). According to traditional standards, the two-tailed P value is equal to 0.4138, and this difference is regarded as statistically insignificant at $p<.05$.

Discussion

In clinical practice, the most important point and the final purpose of maxillary sinus augmentation is the creation of sufficient amounts and high-quality bone for a long-term prognosis is the ultimate goal of maxillary sinus augmentation. Del Fabbro and colleagues 71 demonstrated that the residual bone height is thought to be one of the most important parameters determining implant survival rate, despite the fact that the majority of experts acknowledge that the interpretation of these results is challenging. The initial height of the ridge at the location of future implantation heavily influences the choice of bone augmentation in the posterior maxilla. The stability of the implant will be put at risk if the height of the remaining bone is less than 5 mm. 72 The 2 step open sinus lift approach with or without grafting is the method that offers satisfactory and reliable results. Some studies proved deficient bone formation near the elevated sinus membrane in graftless sinus lifting 73,74. On the controversy, Fuerst *et al*. reported that after sinus grafting with allogenic and xenogenic graft material prior to implant placement in mini pigs 75, bone formation was induced in the region adjacent to sinus membrane. Hence, even though autogenous bone graft remains the gold standard material for promoting new bone regeneration, sinus floor elevation with alternative biomaterials is currently favoured for numerous reasons, including lower morbidity, lower resorption, and unrestricted availability. Bovine bone that has undergone deprotection is usually regarded as the second-best option and the most popular biomaterial for sinus transplantation. Its behavior has been studied and tested for years with positive long-term outcomes 76, 77. Yet keeping in mind the drawbacks of xenografts, such as the danger of disease transmission and the host immune system's response. Some patients also reject it owing to religious objections or because it conflicts with their way of life (e.g. vegetarians) synthetic, alloplastic biomaterials Despite years of research and successful usage in sinus augmentation, relatively few and frequently underpowered trials 80–82 have directly compared alloplasts to xenografts in a split-mouth design. In the current work, bovine bone xenograft was compared to nano-hydroxyapatite. In recent published animal and clinical studies 83,84, nano-crystalline hydroxyapatite bone replacement material has been successfully introduced for augmentation treatment. It appears to be able to induce osteoblast migration, adhesion, and proliferation inside the pore network as well as to promote angiogenesis inside85.

The outline of the sinus on the CT scan, which also aids in measuring the thickness of the lateral wall of the antrum, is used to determine the size and design of the antrostomy. The osteotomy/ostectomy window should be circular, oval, or rectangular with rounded edges, and it should extend 2-3 mm from the anterior limit of the maxillary sinus and 2-3 mm above the maxillary sinus floor. 86,87. In order to reduce the likelihood of the underlying schneiderian membrane being torn by the sharp edges on the bony window, Shalu *et al*. 88 demonstrated that an oval osteotomy is preferred over a rectangular or trapezoidal osteotomy. In the current investigation, a big rectangular osteotomy was performed to create a space for the titanium membrane, which had rounded edges, and to allow for instrumentation.

Summary

The current study examined the utilization of deprotenized bovine bone to Nano HA (Nanostreams) as a predictable grafting material for two-stage sinus floor augmentation (Tutogen)

Twenty patients, aged between 34 and 52 years, of both sexes, were included in this investigation. With remaining bone height less than 5 mm, lateral window sinus lifting was suggested for all patients. For the purpose of keeping the elevated Schneiderian membrane in place, a titanium micro mesh was inserted into the sinus and fastened to the lateral

wall. The twenty patients were split into two groups; the first group (The test group) received a graft made of nano-hydroxyapatite (Nano streams), while the second group received a graft made of deprotened bovine bone (Tutogen), which served as a control. The lateral window was covered with an absorbable collagen membrane. All patients underwent an immediate and six-month postoperative CBCT, and the height of the average vertical ridge height was determined by CBCT at six months after surgery.

The Nano HA group measured 12.1 mm +/- 1.53 mm and the DBB group 12.6 mm +/-1.11mm. In the Nano HA group, the average new bone percentage was 29.84% -/+ 6.7%, while in the DBB group, it was 34.73% -/+ 7.9%. There was no discernible difference between the two groups in terms of bone height gain and the proportion of new bone creation, according to statistical analysis.

Conclusion

The following conclusions can be drawn from the current study's findings

1. Six months after healing, both NHA and DBB resulted in the production of a regenerated new bone, with no statistically significant difference in the new bone height or the new bone formation percentage.
2. NHA can be considered a good grafting material in situations where sinus floor augmentation is necessary to provide room for dental implants.
3. Research with bigger samples and longer follow-ups are advised to evaluate the newly produced bone's long-term durability.

Conflict of Interest

Not available

Financial Support

Not available

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