Sinus Lift Using a Nanocrystalline Hydroxyapatite Silica Gel in Severely Resorbed Maxillae: Histological Preliminary Study

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ABSTRACT

Purpose: The aim of this preliminary study was to evaluate histologically a nanocrystalline hydroxyapatite silica gel in maxillary sinus floor grafting in severely resorbed maxillae.

Materials and Methods: A total of 16 consecutive patients scheduled for sinus lift were recruited during this study. Patients were randomly divided in two groups, eight patients each. In both groups, preoperative residual bone level ranged between 1 and 3 mm (mean value of 2.03 mm). No membrane was used to occlude the buccal window.

Second surgery was carried out after a healing period of 3 months in Group 1 and 6 months in Group 2. Using a trephine bur, one bone specimen was harvested from each augmented sinus and underwent histological and histomorphometric analysis.

Results: Histological analysis showed significant new bone formation and remodeling of the grafted material. In the cores obtained at 6 months, regenerated bone, residual NanoBone, and bone marrow occupied respectively 48 ± 4.63%, 28 ± 5.33%, and 24 ± 7.23% of the grafted volume. In the specimens taken 3 months after grafting, mean new bone was 8 ± 3.34%, mean NanoBone was 45 ± 5.10%, and mean bone marrow was 47 ± 6.81% of the bioptical volume.

Conclusions: Within the limits of this preliminary prospective study, it was concluded that grafting of maxillary sinus using nanostructured hydroxyapatite silica gel as only bone filler is a reliable procedure also in critical anatomic conditions and after early healing period.

KEY WORDS: early loading, histological analysis, nanocrystalline hydroxyapatite, sinus lift

INTRODUCTION

The maxillary sinus floor augmentation technique is widely used in the treatment of resorbed posterior maxilla. Although the use of autogenous bone, as blocks or particulate form, has been considered for a long time the gold standard in terms of grafting material,[1,2] much attention has been paid to the use of bone substitute. When harvesting autologous bone, in fact, donor site morbility[3] has to be taken into consideration. Additional disadvantages are the limited availability and the tendency to resorption.[4]

For this reason, a number of bone substitutes have been evaluated in experimental and clinical studies, such as demineralized freeze-dried bone allograft,[5] bovine bone matrix,[4] resorbable and nonresorbable hydroxyapatite,[6,7] composite bone graft including platelet-rich plasma[8] and tricalcium phosphate.[9]

NanoBone® (Artoos, Rostock, Germany) is a recently developed grafting material consisting of nanocrystalline hydroxyapatite granules embedded in a silica gel matrix. Because of the open SiOH or SiO groups of polysilicic acid, this nanostructured biomaterial presents an extremely large internal surface (about 84 m^2/g). Furthermore, the very rough granule surface creates an interconnecting porous structure ranging from μm to mm dimensions.

Using minipig critical-size defect model, Henkel and colleagues[10] showed a significant higher rate of bone formation when compared to other hydroxyapatite (HA) and TriCalcium Phosphate (TCP) materials or gelatine sponges and an 8 months complete resorption after

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implantation. Moreover, histological and immunohistochemical investigations revealed phenomena of osteoconduction, osteoinduction, and early remodeling.[11]

Further clinical investigation on the biological behavior demonstrated that NanoBone has osteoconductive and biomimetic properties and is integrated into the host's physiological bone turnover at a very early stage.[12] In fact, new bone formation was histologically documented just 3 months after a guided bone regeneration (GBR) procedure.

According to the histological findings of this last paper, the current preliminary study was designed to evaluate the quantitative extent of osteogenesis obtained with a nanostructured hydroxyapatite in maxillary sinus floor grafting after 3 or 6 months of healing. To better assess the Nanobone capability to regenerate bone, experimental protocol was designed selecting patients with very resorbed alveolar crest. Furthermore, membrane was not used to cover lateral sinus window.

MATERIALS AND METHODS

Study Design and Patient Selection

One private dental center consecutively recruited 16 patients scheduled for implant-supported restoration in the posterior maxilla with sinus augmentation procedure.

All patients were in general good health. They were informed about the procedure and were required to sign a consent form.

The inclusion criterium was a residual bone crest (distance between sinus floor and bone crest) ranging between 1 and 3 mm in height.

The exclusion criteria were: sites with acute infection, a full mouth plaque score and a full mouth bleeding score > 25%, schneiderian membrane acute infections or chronic sinusitis, allergies with respiratory component, smokers with >10 cigarettes per day, a history of bisphosphonate therapy, uncontrolled diabetes (HbA1c > 6%, glycemic level > 110 mg/dl), and pregnancy or lactating.

After surgical procedure, patients were randomly divided in two groups, eight patients each:

- Group 1: patients underwent a healing period of 3 months.
- Group 2: patients underwent a healing period of 6 months.

All subjects included in the study were randomly assigned to one of the two treatment regimens (reentry procedure 3 or 6 months after first surgery). Random assignment was performed according to predefined randomization tables. Assignment was performed using a sealed envelope after first surgery.

The present study was performed following the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

Preoperative and Postoperative Medication

Patients underwent a preoperative digital panoramic examination and computerized tomography scan, which were required to investigate antral anatomy.

One week before surgical procedure, full mouth professional prophylaxis appointment was scheduled.

Patients were covered with 1 g amoxicillin/clavulanate 1 day prior to surgery and continued with 2 g per day for 6 days.[13] Penicillin-allergic patients received 450 mg clindamycin. Just before surgery, patients underwent an oral hygiene and then a 3 minute mouth rinsing with 0.2% chlorhexidine gluconate.

Surgical Technique

The sinus area was prepared under local anesthesia, as described by Boyne and James.[1] After lateral window osteotomy, the sinus mucosa was elevated, taking care not to lacerate.

Then the grafting material (NanoBone, Artoos) was placed and meticulously condensed.

According to Del Fabbro and colleagues,[14] in case of extremely resorbed sinus floor, implant placement was not recommendable. In such critical cases, maintaining implant primary stability and angulation is difficult. Therefore, a two-stage procedure was performed.

No membrane was used to close up the buccal window.

The oral mucosa was then sutured with 5.0 resorbable interrupted sutures.

Patients were instructed to avoid blowing their noses for at least 7 days after surgery and to cough or sneeze with an open mouth to prevent increased pressure in the operated sinus.

Second-Stage Procedure

Second-stage surgery to insert the implants was performed 3 months in Group 1 and 6 months later in Group 2 after sinus lift procedure, following randomization.

The implant site osteotomies were performed using a 2 mm inner diameter trephine, and all retrieved grafted bone specimens underwent histological and histomorphometric analysis.

To ensure a complete grafted-material healing, implant restoration was performed 9 months after first surgery in both groups.

Histological Processing

Undecalcified specimens were prepared for light microscopy by the method of Donath and Breuner.[15] Briefly, the grafted biopsies were fixed in 10% formalin/0.1 M phosphate-buffered saline solution (pH 7.4) at room temperature, dehydrated by increasing ethanol concentrations with agitation and vacuum, and embedded in Kulzer Technovit 7200 VLC® (Bio-Optica, Milano, Italy). The cores were sliced longitudinally and subsequently reduced by microgrinding and polishing to an even thickness of 40 µm (Micromet & LS2®, Remet, Bologna, Italy). The sections were mounted on plastic slides, stained with toluidine blue/pyronine G (Sigma-Aldrich, St. Louis, MO, USA), and observed using a Nikon light microscope (Eclipse E600®, Nikon, Tokyo, Japan) equipped with a calibrated digital camera (DXM1200®, Nikon).

Histomorphometry

For histomorphometric analysis, the same sections photographed at a total microscopic magnification of 40× were examined. The volume fractions (V_V) of NanoBone (V_VN), of newly formed bone (V_VB), and of bone marrow and/or connective tissue (V_VC) were calculated by differential point counting according to the Delesse formula:

$$V_V = P_P \quad (1)$$

The computer automatically generated a simple 100-point square lattice system, which was displayed on the television color monitor, directly superimposed on the microscopic field with a systematic sampling. The number of hits containing new bone, grafted particles, or marrow spaces was separately divided by the total number of possible intersections and thus expressed in percentage values representing the volume density of these three components. For each histomorphometric parameter, mean and standard deviations were calculated for the two groups of biopsies (3 months and 6 months postgrafting).

RESULTS

Clinical Observations

A total of 16 patients (eight women and eight men) was treated. The mean age was 56.2 years (ranged 39–86 years).

Preoperative residual bone level ranged between 1 and 3 mm (mean value of 2.03 mm). No statistically significant difference between the two groups in patients' age, sex, and preoperative bone level was found.

The healing period following sinus augmentation was without complication for all patients. Minor nosebleeds occurred in one case. No clinical symptom of maxillary sinusitis occurred in any of the 16 patients.

Histological Outcomes

The specimens harvested at 3 months postgrafting showed large amounts of nonmineralized connective tissue and several residual grafted particles with a homogenous distribution through the histological section (Figure 1a).

Nondegraded granules of hydroxyapatite were surrounded by strands of connective tissue or by an osteoid-like matrix as a sign of early desmal osteogenesis forming woven-bone (see Figure 1b). Interfaces between granules and regenerated bone were intensively stained in most specimens with some multinucleated osteoclast-like cells next to the NanoBone surface (Figure 2), representing stage II of the NanoBone osteogenic process.

On average, regenerated bone, remnants of NanoBone, and bone marrow/soft connective tissue occupied respectively 8% (SD 3.34), 45% (SD 5.10), and 47% (SD 6.81) of the bioptical volume.

Histological examination of the biopsies taken 6 months after grafting gave significant formation of new bone with a prevalent woven-bone structure and some lamellar portions (Figure 3).

An intimate contact was visible between regenerated bone and NanoBone with multiple areas of bone remodeling and graft resorption (Figure 4). In several specimens, a dense extracellular matrix with small blood vessels is invading the intergranular space, thus allowing the entrance of osteoblast-like cells that form new bone and remain incorporated inside (Figure 5).

Mean regenerated-bone density was $48 \pm 4.63\%$, residual NanoBone amounted to $28 \pm 5.33\%$, and bone marrow was $24 \pm 7.23\%$.

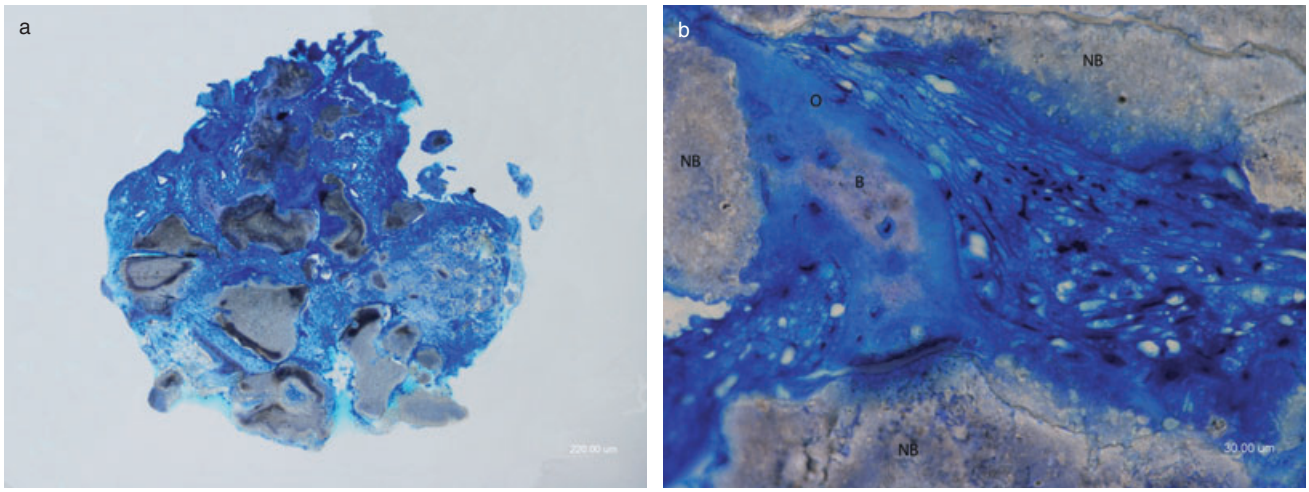

Figure 1 (A) Histological section stained with toluidine blue/pyronine G. Overview of one grafted specimen retrieved at 3 months. Multiple remnants of NanoBone porous particles and small areas of new bone are surrounded by connective tissue (×40 total magnification). (B) Particular of image (A) with a larger magnification (×400). Osteoid (O) is coating a trabecula of regenerated bone (B) interconnecting NanoBone particles (NB). Dense fibrous and well-vascularized connective tissue filled intergranular spaces.

DISCUSSION

This preliminary study demonstrated the possibility of achieving bone regeneration in maxillary sinuses previously grafted with a nanostructured hydroxyapatite starting from 3 months of healing.

Maxillary sinus lift procedures with autogenous bone grafting or allografts and implant placement have been extensively documented and reviewed. Although most authors admit that the interpretation of these results are difficult, Del Fabbro and colleagues[14] showed the residual crestal bone height as one of the most critical factors influencing implant survival rate.

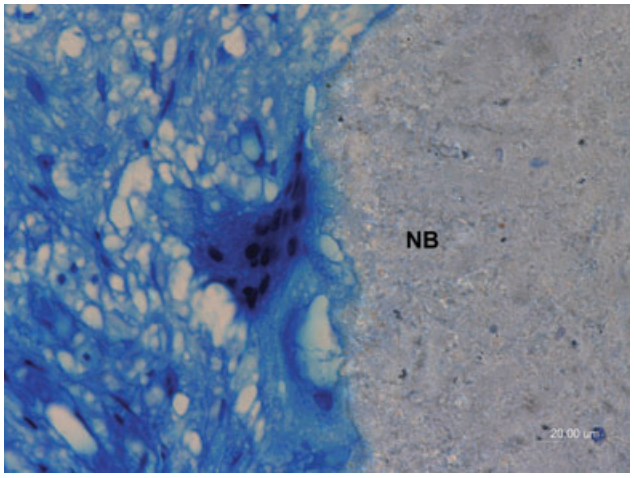

Figure 2 Histological section stained with toluidine blue/pyronine G. Intense cellular activity with multinucleated cells of NanoBone (NB) remodeling in one biopsy at 3 months after grafting (×600 total magnification).

Dental implant placement associated with augmentation of the sinus floor in a severely atrophic maxilla can be performed in one or two surgical stages, depending on the height of the residual alveolar bone. In a one-stage procedure, a minimum base height of 4 to 5 mm is recommended for adequate implant stabilization and parallelism. A two-stage approach is performed when there is insufficient residual bone. This allows healing of the graft material for future implant sites.

Regarding the correct healing time, reviews assumed that an acceptable healing period for grafted sinus procedures ranged between 6 and 9 months.[16,17] According to the literature, this study was performed using a two-stage approach, testing histologically the regenerated bone quantity after 3 and 6 months post-grafting with a nano-sized hydroxyapatite.

Nanocrystalline hydroxyapatite bone substitution material has been successfully introduced for augmentation treatment in recently published animal and clinical studies.[18–20]

The nanostructured hydroxyapatite investigated in the present study is embedded in a highly porous matrix of silica gel. The nanocrystals produce a large, bioactive surface (110 m^2/g) and present a microporosity size ranging from 10 to 20 nm. This configuration seems to be able to induce migration, adhesion, and proliferation of osteoblasts inside the pore network and to promote angiogenesis inside.[12] These events could explain bone formation also at a very early stage, and its rapid maturation was demonstrated histologically in this study.

Figure 3 Histological section stained with toluidine blue/pyronine G. Overview of one grafted specimen retrieved at 6 months. NanoBone residual porous particles are interconnected by newly formed bone and by dense soft connective tissue. A large marrow space with several vessels is noticeable in the center of the image. Native bone components are visible in the first 1 to 2 mm of the coronal portion of the specimen (×20 total magnification).

The current histological analysis revealed the presence of newly formed bone and residual particles of NanoBone that appeared to be partially resorbed and substituted by regenerated bone.

The present histomorphometric data are comparable with the report by Scarano and colleagues.[21] In 16 maxillary sinuses grafted with highly porous hydroxyapatite at 6 months of healing, in fact, they found $32 \pm 2.5\%$ of newly formed bone, $40 \pm 1.6\%$ of marrow spaces, and $34 \pm 1.6\%$ of residual hydroxyapatite.

The current findings are very encouraging, considering that the present biopsies were all retrieved from highly resorbed alveolar crests (1–3 mm) with a minimum content of native bone.

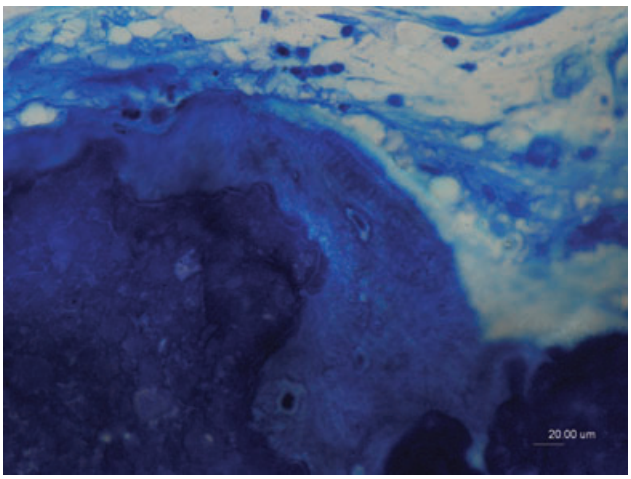

Figure 4 Histological section stained with toluidine blue/pyronine G. The grafted particles are incorporated into the regenerated bone (×600 total magnification).

In addition, similar values of new bone fractions were obtained after 6 months of healing in surgical sites grafted with β-tricalcium phosphate and deproteinized bovine bone. [9,21,22]

Nowadays, 6 months is considered the optimal period of a bone graft healing. In fact, the osteogenic process is completed in the first 6 months, and further extension of the follow-up might increase bone-remodeling activity with progressive bone resorption. Besides, in their review, Merkx and colleagues[23] showed adequate new bone formation 3 to 4 months after composite graft implantation.

Therefore, two different periods of NanoBone healing (3 and 6 months) were analyzed in the present

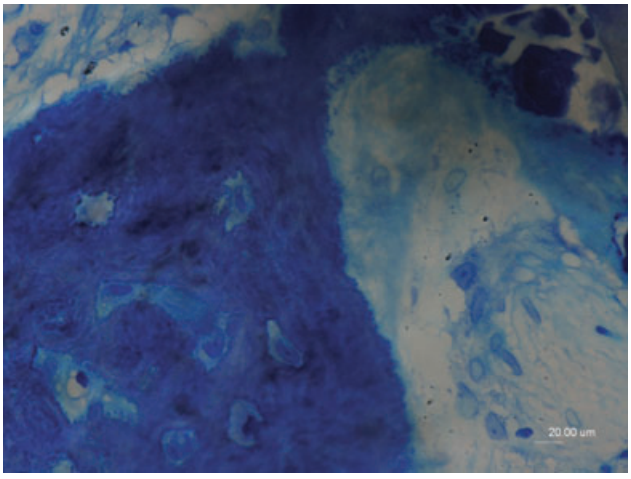

Figure 5 Several osteogenic cells are incorporated into the newly formed bone; a woven-bone structure is present in the intergranular spaces (×600 total magnification).

preliminary study. Comparing the two groups of biopsies investigated, a massive increment of the new bone percentage volume was found. An interindividual variability was found in the regenerated bone fractions within both groups of biopsies, reflecting different stages of granule osteogenesis also in distinct areas of the same specimen as depicted by Götz and colleagues.[12] The tendency to an early maturation of the regenerated bone is highlighted also by the rapid decrease of residual NanoBone.

As demonstrated immunohistochemically,[12] and by scanning electronic microscope and energy-dispersive X-ray analysis,[24] this fast turnover could be correlated to the SiO_2 gel matrix of NanoBone, which is degraded and substituted by an organic matrix, and to the hydroxyapatite nanoporosity, which would allow bone matrix proteins to adhere and promote differentiation of osteoblast precursor cells.

However, the factors influencing the different behavior (stages of osteogenesis and rates of graft resorption) of the NanoBone augmented areas need to be investigated in the future to correlate the stages of osteogenesis with different time points to individual healing-bone patterns.

In their systematic review, Wallace and Froum[25] indicated membrane placement over the lateral window as an important factor to improve regenerated bone quality. An absorbable collagen membrane placed on the buccal sinus wall, in fact, seemed to prevent graft from soft tissue invasion, which would reduce the amount and the quality of the de novo-formed mineralized tissue.[26,27] Furthermore, in a bilateral randomized controlled trial with the presence or absence of a collagen membrane over the window being the only variable, Tarnow and colleagues[28] reported a vital bone formation of 25.5% (SD 14.5) when a membrane was utilized, and 11.9% (SD 7.9) when a membrane was not placed over the lateral window.

In the present study, although no membrane was used to occlude the buccal bone access, histological outcomes were superimposable to the ones listed below.

Within the limits of this preliminary prospective study (limited number of patients), the observed nanocrystalline hydroxyapatite silica gel seems be effective also in critical conditions such as absence of membrane on the buccal wall and low residual bone height in maxillary sinus lift procedures. The finding of newly formed bone after 3 months of healing, although in limited quantities, could lead to clinically highlight the potential of this grafting biomaterial even in the very early stages of bone maturation as already suggested by Götz and colleagues.[12]

However, obtained results are to be confirmed with further studies using a split-mouth design or clinical randomized controlled trials comparing nanocrystalline hydroxyapatite to autogenous bone, focusing on implant survival rate.

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CONFLICTS OF INTEREST STATEMENT

The authors have declared no conflicts of interest. [Correction added after online publication 23 October 2009: Conflicts of Interest Statement added.]

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