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Original Article

Histological and histomorphometric evaluation of the synthetic biomaterial Natix® in horizontal reconstruction of alveolar ridge

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ABSTRACT

Background: Following loss of teeth, atrophy of alveolar ridge of the jaws is a substantial problem and unintended outcome that compels clinicians to perform bone reconstruction ahead of implant placement. Although autogenous bone is recommended as the gold standard in bone reconstruction, an invasive second surgery harvesting a limited volume of bone (from intraoral source) has led to a significant approach to the use of synthetic bone substitute materials. The aim of this study was to evaluate the histologic and histomorphometric properties of porous titanium granules (Natix®) used in horizontal reconstruction of alveolar ridge before implant placement.

Materials and Methods: In the present quasi-experimental clinical trial, four patients (three females and one male) needed horizontal bone augmentation on ten areas of edentulous mandibular ridge before implant treatment. During surgery, the buccal aspect of edentulous ridge was augmented by Natix®, covered by resorbable membrane (Cytoplast®). After 8 months, 10 core biopsies were obtained.

Results: In histological study, no foreign body reaction at the site of the newly formed bone or around the biomaterial residue was observed. Newly formed bone was fully vital with large lacunae containing osteocytes. In 60% of cases, connective tissue was observed at the biomaterial – new bone interface. In histomorphometric study, mean percentage of bone formation was $40.56\% \pm 19.83\%$ and mean bone trabecular thickness was $39.98 \pm 17.54 \mu$.

Conclusion: Despite acceptable histological and histomorphometric bone formation findings, in clinical terms, no increase was created in the horizontal dimension. Thus, it seems that application of this biomaterial in horizontal reconstruction of alveolar ridges with noncontained defects is inappropriate.

Key Words: Augmentations, alveolar ridge, bone regeneration, cytological techniques, histology, porosity, titanium

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INTRODUCTION

Local defects of the alveolar ridge resulting from trauma, atrophy of the ridge or periodontitis, reduce the horizontal dimensions of the alveolar bone, which could interfere with the process of dental implant

placement. Hence, following loss of teeth, decrease seen in the horizontal dimension of the alveolar ridge, is a substantial problem and unintended outcome that compels clinicians to perform bone

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reconstruction ahead of implant treatments. Which could, therefore, interfere with implant-prosthetic rehabilitations.^[1] Autogenous bone has been the only source of osteogenic cells to date and thus is considered the gold standard for oral reconstruction. Although autogenous bone is recommended as the gold standard in bone reconstruction, a second invasive surgery producing limited volume of bone (from intraoral source) or residual pain, and cosmetic disadvantages created by harvesting autologous bone from the iliac crest has led to a significant approach toward the use of synthetic bone substitute materials, from allogeneic, synthetic, and xenogenic sources as osteoconductive materials to overcome limitations and complications associated with autogenous bone grafts.^[2,3] Most bone substitute materials in use today are hydroxyapatite based such as bovine hyaluronic acid (HA) or synthetically produced HA; and despite the slow rate of absorption, they are absorbed either too much or too little.^[4] Natix® is a new bone substitute material, made of commercially pure titanium that is resistant to atrophy with good coagulation properties as an osteoconductive material in reconstruction of medium to large lesions and is favored because of ease of application (granule binding together during reconstruction). More importantly, results obtained from *in vitro* studies on animal and human samples for using this material in surgical orthopedic fixation of the femoral shaft and inducing bone formation have been promising.^[5] Since there are no reports of clinical application of this material in horizontal alveolar ridge reconstruction, thus, the aim of this study was histological and histomorphometric evaluation of this biomaterial in horizontal reconstruction of alveolar ridge.

MATERIALS AND METHODS

In the present quasi-experimental clinical trial, four patients (three females and one male) were selected. They needed ten areas of horizontal bone augmentation, were aged between 30 and 50 years, and applied for implant treatments in edentulous mandibular ridge with sufficient bone height, but insufficient buccolingual width and had at least one area in need of treatment [Table 1]. Widths between 2 and 5 mm, with clinical measurement of ridge thickness of soft tissue using three-dimensional (3D) Bone Caliper (Blue and Green Inc., Canada) were selected [Figure 1a]. The local ethic committee accepted the study.

All patients received 600 mg clindamycin ½ h before ridge augmentation surgery. After local anesthesia, a crestal incision was made in the edentulous ridge to reflect a full-thickness flap to access buccal ridge bone. Then, all soft tissue was removed from the bone [Figure 1b], and the cortical buccal bone was decorticated with a 2 mm round bur at 4 mm intervals [Figure 1c]. After periosteal fenestration for tension-free flap closure, Natix® biomaterial (Tigran Inc., Swiss) with granular size of 500–1000 µ was placed at the site of the bone defect after mixing with blood and saline [Figure 1d].

The augmented area was then covered with absorbable Cytoplast® Membrane (Novatech Inc.,

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Partially edentulous patients	Any contraindications for elective oral surgical procedures
Alveolar ridge defects requiring lateral augmentation in at least one area before implant placement	Immunosuppressive or anticoagulant medication
Age from 30 to 50 years	Systemic or local bone diseases
High compliance and high oral hygiene status	Uncontrolled diabetes
Written informed consent from each patient	Minor compliance and no ability or willingness to maintain oral hygiene

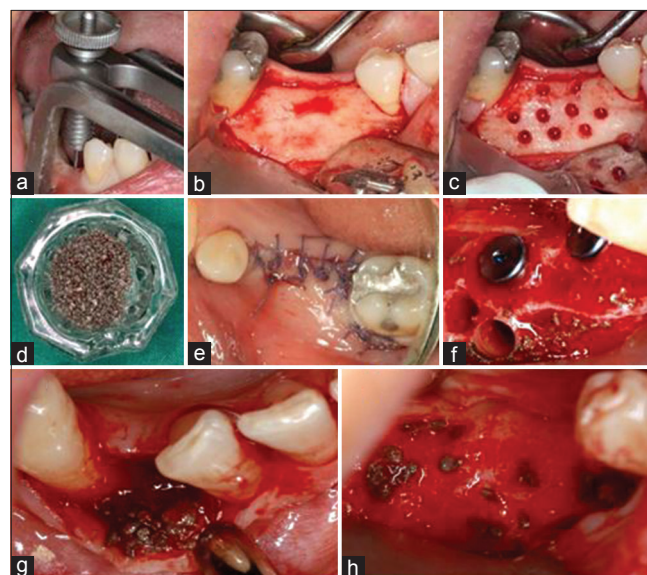


Figure 1: (a) Ridge width determination by three-dimensional bone caliper. (b) Flap reflection and exposing buccal aspect of the ridge. (c) Decortication of buccal ridge aspect. (d) Porous titanium granules. (e) Suture of the flap with Vicryl 4-0. (f) Core biopsies obtained from lateral aspect of the ridge. (g) Porous titanium granules embedded in soft tissue flap after flap reflection. (h) Remaining porous titanium granules attached to bone after flap reflection.

USA). The tension-free flap was sutured using Vicryl® 4-0 (Ethicon Inc., USA), with horizontal mattress and interrupted sutures [Figure 1e]. After the surgery, clindamycin 600 mg 3 times per day for 7 days, appropriate analgesic, and Peridex® 0.12% mouthwash (3M ESPE Inc., USA) twice daily for 2 weeks were administered.

After 14 days, sutures were removed, and 8 months later, full-thickness flap was elevated in preparation for the implant surgery. Depending on the edentulous ridge space that was scheduled for one or two implants, one or two bone core biopsy samples were prepared by 3 mm Trephine bur from lateral reconstructed ridge side and SPI dental implants (Thommen Medical Inc., Switzerland) were placed according to the manufacturer's recommendations [Figure 1f]. Samples were sent to the laboratory in 10% formalin for histological and histomorphometric studies.

Histological study

The biopsy samples were maintained in 10% formalin for at least 10 days for full fixation. They were then kept in 10% formic acid for decalcification for 1 week and daily examined for softness required to cut with microtome (Leica Inc., USA). To neutralize the acid, samples were submerged in lithium bicarbonate 20% solution for 5 min. Each sample was marked with a number. Eventually, bone samples were cut longitudinally in two parts, and the cut edges indicating middle of the bone were marked with India ink. Samples were then placed in different degrees of alcohol for serial dehydration and placed in paraffin blocks from the marked side. Seven slices of 5- μ thickness were prepared from each sample and stained with H and E and examined under the optical microscope (Olympus BX41, Tokyo, Japan).

In histological study, the following parameters were measured and recorded by the pathologist: (1) inflammation level, (2) presence or absence of reaction to a foreign body (giant cell and granulomatous reaction), (3) bone vitality (presence or absence of osteocyte in lacunae), (4) bone trabecular thickness, (5) bone-biomaterial contact form (presence or absence of connective tissue between bone fragments). The different grades of inflammation^[6] are presented in Table 2.

Histomorphometric study

All prepared sections from grafted areas were photographed using a digital camera with the Olympus microscope at $\times 40$ magnification, and resulting

images in JPEG format were entered to SIS LS Starter software (Olympus Inc., Japan). Then, bone formation areas were chosen, and bone formation percentage was found from the ratio of bone area to the overall area of the image. In histomorphometric study, bone trabecular thickness was determined.

Furthermore, the number of blood vessels was evaluated in three microscopic fields with $\times 10$ magnification and divided into three grades [Table 3].^[6,7] At no stage was the pathologist aware of the biopsy content so that any bias could be prevented in interpretation of histological-histomorphometric results. For accurate measurement in this method, seven sections were selected from each biopsy sample, and mean value was considered as the final result. After obtaining the data, statistical analysis was expressed in the form of descriptive indicators (mean, ratio, and so on).

RESULTS

In histological study, no foreign body reaction or presence of multinucleated giant cells at the site of the newly formed bone [Figure 2a] or around the biomaterial residue was observed [Figure 2b]. In all seventy examined samples, newly formed bone was fully vital with large lacunae containing osteocytes [Figure 2c]. In 60% of cases, connective tissue was observed at the biomaterial – new bone interface [Figure 2d and e].

In the histomorphometric study of the prepared samples (seven images of each sample), using SIS LS Starter software (Olympus Inc. Tokyo,

Table 2: Different grades of inflammation

Grade of inflammation	Characteristics
Grade 0	Absence of inflammatory cells
Grade 1	Presence of a few scattered inflammatory cells (mild)
Grade 2	Up to 5-10 inflammatory cells (focal)
Grade 3	10-50 inflammatory cells (focal)
Grade 4	>50 focal inflammatory cells (severe inflammation)

Table 3: Different grades of the quantity of blood vessels in microscopic fields with $\times 10$ magnification

Grade of Inflammation	Number of Blood vessels
Grade 0	<3 blood vessels observed in the field
Grade 1	Between 3 and 5 blood vessels observed in the field
Grade 2	>5 blood vessels observed in the field

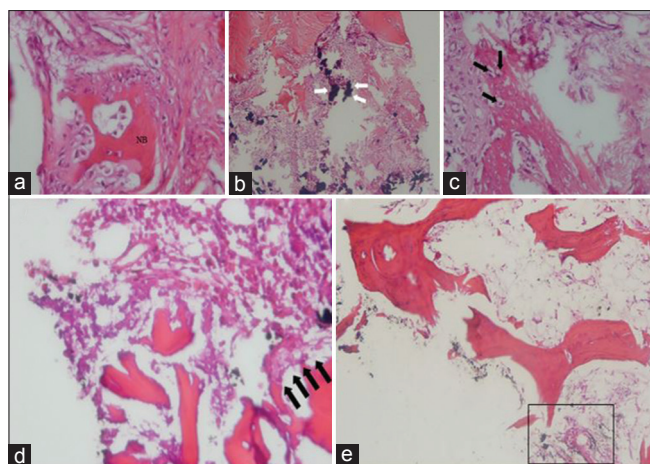


Figure 2: (a) Histologic appearance of newly formed bones. NB = newly formed bone; ($\times 40$; H and E). (b) Residual bone material presents at the bone-material interface. White arrows = Porous titanium granules ($\times 40$; H and E). (c) Black arrows = Osteocytes in lacunae ($\times 40$; H and E). (d) Black arrows = Bone-biomaterial interface ($\times 40$; H and E). (e) Rectangular box = Grade 3 inflammation at the bone-biomaterial interface ($\times 10$; H and E).

Japan), mean percentage of bone formation was $40.56\% \pm 19.83\%$, and mean bone trabecular thickness was $39.98 \pm 17.54 \mu$ (Grade 2).

DISCUSSION

In recent years, porous titanium granules (Natix®) have been used in orthopedic surgical fixation of the femur, sinus augmentation procedures, and dehiscence and fenestration defects formed around implants due to peri-implantitis. Since no reports were found of clinical application of Natix® as an osteoconductive material in horizontal reconstruction of alveolar ridge, thus this biomaterial was used for horizontal reconstruction of the alveolar ridge in this study.

According to histological examination, in 70% of samples, no inflammation or mild inflammation was observed around the biomaterial residue. No foreign body reaction or presence of multinucleated giant cells at the site of newly formed bone or around the biomaterial residue was observed. In all seventy examined samples, newly formed bone was fully vital. In 60% of cases, connective tissue was observed at the interface between biomaterial and newly formed bone, and this was consistent with patient's clinical state in the second stage surgery, during core biopsy preparation, implant insertion which in most cases, Natix® biomaterial easily separated from the bone and

was connected to the flap at the time of second stage surgery [Figure 1g and h].

In histomorphometric study, mean newly formed bone area and bone trabecular thickness were $40.56\% \pm 19.83\%$ and $39.98 \pm 17.54 \mu$, respectively. With regard to the lack of bonding between Natix® and the bone in some areas, there are several possible reasons that are discussed below. The protective membrane in guided bone regeneration (GBR) should have the following properties: tissue integration, cell occlusive properties, ability to maintain space, and ease of clinical application.^[8] One of the disadvantages in application of absorbable membranes in GBR is limited control over absorption time that should ordinarily be able to maintain structural integrity for more than 6 months, but upon placement in tissue and exposure to enzymes related to macrophages and neutrophils, membrane quickly dissolves and loses its structural integrity.^[9] For increased survival of the membrane, cross-linking techniques have been used.^[10] According to histological and immunohistochemical studies on cross-linked membranes, it has been shown that these membranes are somewhat cytotoxic, which impairs binding and proliferation of human osteoblasts and delays blood vessel invasion of areas under the membrane, thereby restricting tissue integration rate.^[11,12] For bone regeneration, it is necessary that transmembranous angiogenesis should occur to transform osteogenic cells in the form of pericytes to the area of interest.^[13,14] Furthermore, the problem of reduced water absorption and increased membrane rigidity affects its clinical application and could also affect treatment outcome, and this was evident in the Cytoplast® membrane used in this study.^[15] One way to overcome shortcoming related to these membranes is the use of noncross-linked membranes in double layers, which survives for 6 months. This increased thickness in collagen content increases transmembranous angiogenesis of the membrane, thereby creating a positive impact on bone regeneration.^[16] On the other hand, according to studies, condensation and excessive application of graft material at the surgical site are considered a biological disadvantage since bone regeneration requires new in-growth of blood vessels through spaces between granules; otherwise, angiogenesis and bone formation will not occur.^[17]

Turner *et al.*, in a study on a dog model, conducted a histological examination of bone growth between titanium granules used as femur joint replacement.

A canal was created in the joint, and granules were vibrated into the intended cavity, and the cavity opening was fixed by titanium plate and screws. Six months later, new bone formation between and around granules was observed.^[18]

Holmberg *et al.* performed ridge splitting operation with nine implants in a patient requiring horizontal reconstruction of the maxilla and filled the space between implants with porous titanium granules; 12 years later when the patient was recalled, the implants had remained stable during this time.^[19]

Jónsson and Mjöberg filled defects caused by fracture of the tibia with porous titanium granules and stabilized the fracture area with internal fixation screws or buttress plate. Clinical results in four patients under study were satisfactory.^[20]

It is known that some local factors can also affect the success of reconstructive treatments such as stability and immobility of the graft.^[1] In comparing the present study with the above studies, success of Natix® could be explained by the fact that in the above studies, treated lesions were of contained type (surrounded by bony walls), which influenced immobility of granules in the operation site as well as bone growth induction, whereas in the present study, bone defect was not a contained type defect and to stabilize granules in place and prevent their displacement, membrane fixation techniques were not used.

Another way to stabilize the graft is application of titanium-reinforced nonabsorbable membranes and immobilizing it in place with screws. However, one of the disadvantages of these membranes is frequent dehiscences in the soft tissue associated with membrane exposure, which causes bacterial contamination and infection of the regeneration area and impaired osteogenesis process.^[21]

Bystedt and Rasmusson (2009) used porous titanium granules for maxillary sinus lift. According to the results, when sinus graft and implant placement were performed simultaneously, porous titanium granules performed well, and survival rate of implants was around 87%, but in the two-stage operation, implants failed.^[22] In some respects, results of the present study are comparable with those of Bystedt's study. In the above-mentioned study, all five implants inserted few months after the sinus augmentation failed. Granules had separated from the site and moved in different directions during osteotomy implant preparation due to lack of proper bone growth between them. This

problem was also observed in the present study; even though the two studies were different in terms of study type (histological or clinical), reconstruction site (maxilla or mandible), type of reconstruction (horizontal or vertical), and reconstruction interval between two surgeries (<6 months against 8 months).

Wohlfahrt *et al.*, using micro-computed tomography technique in histological method, investigated porous titanium granules as graft material in regeneration of one to three wall defects created due to peri-implantitis in an implant. Twelve months later in the second surgery, biopsy was prepared from the intended area. Results showed that granules had integrated well with lamellar and woven bone areas, and in some cases, thin areas of fibrous tissue between new bone and implant surface were observed.^[23]

In the present study, presence of connective tissue at the biomaterial-bone interface was almost similar to Wohlfahrt study. Histological results revealed the presence of fibrous connective tissue at some parts of the implant surface, which before augmentation procedure had a configuration like a one-wall defect. Parts of the graft used for reconstruction of the one-wall defect next to implant surface had been lost due to instability. Although the two studies were different in terms of type of the study (case report or clinical trial), a number of samples (one against ten), biomaterial application technique (on the bone surface or implant surface), and histological study method (optical microscope or electron microscope). In the present study, giant cells or foreign body reaction was not observed in any of the samples, which indicate biomaterial tissue compatibility.^[24]

The newly formed bone was fully vital in all samples and contained large lacunae with osteocyte, which shows the performance of porous titanium granules as an osteoconductive material in bone regeneration. According to histological studies, when lacunae lack osteocytes, bone is considered nonvital.^[24]

Mean area and thickness of new trabecular bone were $40.56\% \pm 19.83\%$ and $39.98 \pm 17.54 \mu$, respectively, and trabecular thickness was in the medium range (Grade 2). In a study by Rokne *et al.* (2003), trabecular thickness of bone in the treated group with Bio-Oss® was reported (medium) Grade 2 in 40% of cases, after 6 months, which is in agreement with the present study,^[25] even though the two studies differed in terms of type, biopsy sampling method, and type of material under study.

It is worth noting that in histomorphometric studies, a two-dimensional image of a 3D space creates limitations in interpreting histological sections of the reconstructed bone areas.^[26] Thus, in addition to the effect of biological factors on thickness of bone trabeculae, technical problems such as direction of preparation of sections in relation to longitudinal axis of defect (vertical or parallel) totally influences the microscopic view obtained and could explain the difference in results of these studies. Furthermore, reported histomorphometric results in various studies must be compared with one another and interpreted cautiously because biopsy sampling in animal studies is different from human studies. Furthermore, bone core samples are different in human studies; some are vertically obtained, and some are horizontally, like in the present study.^[26] Furthermore, the fact that core fully contains the bonded area, or part of the bonded area, is also important. If core biopsy fully contains the bonded area, how much of the core contains native bone, and how much contains bonded area is important in calculation of the quantities mentioned in histomorphometric studies.

Finally, as discussed, in 60% of cases, there was connective tissue at the bone biomaterial junction. As is known, in histological studies, use of optical microscope merely provides information about connection type at the junction (tissue or bone). While ultrastructural information about organization at the junction is only possible with the use of electron microscope.^[27] Thus, use of electron microscope for a better understanding of molecular events in the area is recommended.

CONCLUSION

Given the limitation in the present study, it appears that Natix®, containing porous titanium granules, is a biocompatible and osteoconductive material. Despite acceptable histological and histomorphometric bone formation findings, in clinical terms, no increase was created in the horizontal dimension. Thus, it seems that application of this biomaterial in horizontal reconstruction of alveolar ridge, which are in noncontained defects, is inappropriate. However, for more definitive results, further studies are recommended.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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