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<td>Q1</td>
<td>Please complete and update the reference given here (preferably with a DOI if the publication data are not known): Canullo and Dellavia (in press). For references to articles that are to be included in the same (special) issue, please add the words ‘this issue’ wherever this occurs in the list and, if appropriate, in the text.</td>
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Thank you for your assistance.
Maxillary sinus floor augmentation using a nano-crystalline hydroxyapatite silica gel: Case series and 3-month preliminary histological results

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\begin{abstract}

The aim of this case series is to histologically examine a new hydroxyapatite in sinus lift procedure after 3 months. Ten 2-stage sinus lifts were performed in 10 healthy patients having initial bone height of 1–2 mm and bone width of 5 mm, asking for a fixed implant-supported rehabilitation. After graft material augmentation, a rough-surfaced mini-implant was inserted to maintain stability of the sinus widow. A bioplastic core containing a mini-implant was retrieved 3 months after maxillary sinus augmentation with NanoBone\textsuperscript{®} and processed for undecalcified histology. From the histomorphometric analysis, NanoBone\textsuperscript{®} residuals accounted for the 38.26\% ± 8.07\% of the bioplastic volume, marrow spaces for the 29.23\% ± 5.18\% and bone for the 32.51\% ± 4.96\%. The measured bone-to-implant contact amounted to 26.02\% ± 5.46\%. No connective tissue was observed at the implant boundary surface. In conclusion, the tested material showed good histological outcomes also 3 months after surgery. In such critical conditions, the use of a rough-surfaced mini-implant showed BIC values supposed to be effective also in case of functional loading. Although longer follow-up and a wider patient size are needed, these preliminary results encourage further research on this biomaterial for implant load also under early stage and critical conditions.

\end{abstract}

\section{Introduction}

Clinically, the posterior maxilla often represents a hardly suitable zone for implant placement due to insufficient available bone. Sinus floor elevation was developed to increase needed vertical height to overcome this problem (Del Fabbro \textit{et al.}, 2004; Wallasch and Froum, 2003). Variable augmentation materials and techniques using various bone grafts and bone substitutes have frequently been used to enable placement of posterior maxillary dental implants (Cammack \textit{et al.}, 2005; Ewers \textit{et al.}, 2004; Karabuda \textit{et al.}, 2001; Maiorana \textit{et al.}, 2000).

A newly developed bone grafting substitute consisting of nano-crystalline hydroxyapatite (HA) and nanostructured silica (SiO\textsubscript{2}) (NanoBone\textsuperscript{®}, Artoss, Rostock, Germany) is now available for clinical application. It was described to be osteoconductive and biodegradable in a manner comparable to natural bone remodeling processes (Henkel \textit{et al.}, 2006). Furthermore, clinical investigation has demonstrated that NanoBone\textsuperscript{®} has osteoconductive and biomimetic properties and is integrated into the host physiological bone turnover at a very early stage (Götz \textit{et al.}, 2008). Recent clinical trials have shown that grafting of the maxillary sinus floor using a nano-structured hydroxyapatite silica gel as a bone filler is a reliable procedure in critical anatomical conditions after the early healing period (Canullo and Dellavia, in press).

The aim of this prospective study was to evaluate tissue composition of the augmented maxillary sinus floor 3 months after using a nano-crystalline hydroxyapatite bone substitute. Histological analysis and bone-to-implant contact (BIC) assessment between the grafting material and inserted mini-implant were achieved.

\section{Materials and methods}

\subsection{Patient selection}

All procedures and materials in the present prospective study were approved by the local ethics committee, and all patients provided informed consent.

Ten patients in need of fixed implant-supported prosthesis in the posterior maxillae were recruited for the present study. The patients were in good general health and had a median age of 54

\begin{thebibliography}{1}


\end{thebibliography}
Overview of 1 mini-implant positioned in sinus lift grafted with NanoBone (Fig. 1).

2.2. Surgical procedure

Patients received 875 mg of amoxicillin/clavulanic acid (1 capsule/12 h) 1 day before the surgery and for 6 days. After local anesthesia, a crestal incision was made at the implant site and sulcular at the adjacent teeth if present. Subsequently, a vertical releasing incision was made distally and the muco-periosteal flap was raised. A rectangular or oval-shaped osteotomy was then prepared on the lateral aspect of the alveolar ridge under copious normal saline irrigation. The resulting detached “window” was elevated medially and apically while simultaneously reflecting the sinus membrane.

After adequate reflection, the sinus membrane was inspected for tears and NanoBone® (Artoss) mixed with antibiotic solution (Lincocin 600 mg, Pharmacia Italia S.p.a., Milano, Italy) was placed incrementally at the superior aspect of the sinus and against the medial aspect of the grafted compartment created in the sinus cavity (Yaman et al., 2007). The graft material was meticulously condensed at each stage. A nano-surfaced mini-implant of 1.2 mm diameter and 13 mm in length (PI Branemark Philosophy, Bauru, Brazil) was then positioned to maintain the space opening. This implant, in fact, kept the internally rotated sinus bone door constantly apart from sinus floor, contrasting eventual increased pressure in the operated sinus. Single interrupted sutures were finally used for flap adaptation. This surgical procedure was applied unilaterally for each patient of this study.

2.3. Histological assessments

After a 3-month healing period, a biooptical core containing the mini-implant was retrieved using a 3 mm trephine bur. Samples were immediately immersed in buffered 10% formalin solution with a pH of 7.4 for 5 days at room temperature (Gedrange et al., 2008) and then processed for undecalcified histological analysis according to a previously described protocol (Donath and Breuner, 1982). In brief, all bone cores were dehydrated in ascending grades of ethanol, infiltrated and embedded in acrylic resin using a light polymerization unit (Kulzer Technovit 7200 VLC, Bio-Optica, Milano, Italy). The cylinders were sectioned in the vertical plane using a diamond saw (Micromet, Remet, Bologna, Italy) and the 2 mid-sections were ground and polished to final thickness of 40–50 μm (LS2, Remet, Bologna, Italy), and finally stained with toluidine blue/pyronine G (Sigma-Aldrich, St Louis, MO). Qualitative and quantitative analyses were performed with the aid of a Nikon light microscope (Eclipse E600) equipped with a calibrated digital camera (DXM1200, Nikon, Tokyo, Japan) and a semi-automated computer program (Rhinoceros NURBS modeling for Windows, version 3.0, McNeel, Seattle, WA 98103 USA).

The following morphometric measurements were determined: (1) the bone-to-implant contact, measured as a fraction of the mini-implant length in direct contact with mineralized bone at a total magnification of 100×; (2) the proportion (%) of hard tissue occupied by either new bone, NanoBone® or connective tissue/marrow spaces, estimated by a point counting technique. A lattice comprising 100 test points was superimposed over each histologic section photographed at a total magnification of 40×. The number of grid intersections containing NanoBone® (Artoss), bone (regenerated and native bone, distinctively), and bone marrow were separately recorded, divided by the total number of possible intersections and thus expressed in percentage values representing the fraction area of the 3 histologic components.

3. Results

After 3 months of healing, varying amounts of newly formed bone were found through the specimens (Fig. 1). From the morphometric analysis, NanoBone® (Artoss) residuals accounted for 38.26% ± 8.07% of the extracted bone volume, marrow spaces presented 29.23% ± 5.18% and bone occupied 32.51% ± 4.96%.
bone: 20.64% ± 2.96%, native bone: 11.87% ± 3.27%). Structurally, the new bone was a mixture of woven, maturing woven and lamellar bone with lamellar parallel-fibred structure and Haversian systems becoming apparent when viewed under polarized light (Fig. 2). Marrow spaces among bony trabeculae were wide, highly vascularized and poor in adipocytes. There was no inflammatory reaction around the residual grafted particles, located mostly in the apical portion of the histologic sections as shown in Fig. 1. Nanobone remnants were encapsulated by loose highly vascularized connective tissue with a varying degree of contact with new bone (Figs. 3 and 4). Several implant threads exhibited close contact with the regenerated bone (Fig. 4). Occasionally, a layer of provisional matrix (osteoid) composed by cellular-fibrous connective tissue ongoing mineralization process was observed adjacent to the titanium surface (Figs. 4 and 5). Mean BIC was 26.02% ± 5.46%.

4. Discussion

The results of this study have indicated that NanoBone® (Artoss) could be suitable for maxillary sinus floor augmentation as it proved osteogenic behavior, i.e. bone regeneration, at very early healing stage in critical vertical bone height conditions. This was demonstrated by histological and histomorphometric analyses carried out in the present study, in addition to immunohistochemical, SEM, and energy-dispersive X-ray analyses performed in other basic studies (Gerber et al., 2006; Götz et al., 2008) (Table 2).

In the literature, 6–9 months were considered the optimal period for bone graft healing as the osteogenetic process was considered completed thereafter (Del Fabbro et al., 2004; Wallace and Froum, 2003). However, the present investigation results showed that NanoBone® (Artoss) presented a fast turn-over compared to other biomaterials (Galindo-Moreno et al., 2007; Tarnow et al., 2000; Valentini et al., 2000). This might be correlated to the SiO₂ gel matrix of the material that 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 (Gerber et al., 2006; Götz et al., 2008). Another biomaterial with a likewise fast turn-over and osteoconductive properties is BONITmatrix®, a nanoporous, granular scaffold composed of hydroxyapatite, tri-calcium phosphate embedded in SiO₂. Recently it was shown that BONITmatrix® and heavily fractionised BONITmatrix®, called OSSA NOVA, could stimulate bone regeneration, but OSSA NOVA leads to an accelerated
more comprehensive bone regeneration in comparison to granular BONITmatrix® (Kunert-Keil et al., 2009).

As literature indicates that in instances of severely resorbed maxilla required healing time should be between 6 and 12 months (Del Fabbro et al., 2004; Fugazzotto and Vlassis, 2007; Wallace and Froum, 2003), demonstrated early healing and bone regeneration using NanoBone® (Artox) should encourage longer follow-up and further animal and clinical investigations concerning early implant loading. The current amount of regenerated bone confirms previous histomorphometric data from 8 patients treated with NanoBone® 3 months post-grafting (Canullo and Dellavia, in press).

Regarding the mini-implant surface used in this study, the resulting mean BIC of 26.02% was comparable to that in a canine model by Conner et al. (2003). In that study, mean BIC values were reported as 16.24% for acid etched surfaces and 25.08% for TPS after a 4-month healing period (Conner et al., 2003).

Another animal study reported mean BIC values of 14.3–17.6% for turned implants and 37.3–44.7% for oxidized implants after a 6-month healing period (Palma et al., 2006). Although it is hard to compare animal to clinical studies, the BIC values reported in the present and the quantity of new bone found at 3 months of healing could clinically assess the potential of this grafting biomaterial even at very early stages of bone maturation.

According to Wallace and Froum (2003), the use of collagen barrier membrane to enhance bone regeneration in maxillary sinus augmentation to prevent soft tissue invasion was highly recommended (Becker et al., 1995; Buser et al., 1996). However in the present investigation, the absence of barrier membrane application to occlude buccal bone wall did not influence healing as histological outcomes of bone formation and absence of connective tissue were observed at the mini-implant boundaries (Tarnow et al., 2000).

Finally, the tested material showed good histological outcomes also 3 months after surgery. Under such critical conditions, the use of a rough-surfaced mini-implant showed BIC values supposed to be effective also in case of functional loading. The presented preliminary results encourage further research on this biomaterial in augmenting critical vertical bone conditions and immediate or early implant loading for a longer follow-up period and larger patient cohort size.

Acknowledgement

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Table 2

Histomorphometric measurements in the bone cores. NIJOVA.

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<th>Patient</th>
<th>Nanobone (%)</th>
<th>Bone marrow (%)</th>
<th>New bone (%)</th>
<th>Native bone (%)</th>
<th>BIC (%)</th>
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<td>I</td>
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<td>24.3</td>
<td>17.8</td>
<td>10.1</td>
<td>25.36</td>
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<tr>
<td>II</td>
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<td>31.4</td>
<td>18.3</td>
<td>5.2</td>
<td>18.50</td>
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<td>19.4</td>
<td>22.2</td>
<td>11.1</td>
<td>31.78</td>
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<tr>
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<td>33.3</td>
<td>25.1</td>
<td>10.4</td>
<td>24.32</td>
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<td>V</td>
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<td>Mean</td>
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<td>3.27</td>
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