

# Maxillary Sinus Augmentation with the Xenograft Bio-Oss and Autogenous Intraoral Bone for Qualitative Improvement of the Implant Site: A Histologic and Histomorphometric Clinical Study in Humans

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*The aim of the present clinical study was to determine, through histologic and histomorphometric investigations of human bone specimens, whether the addition of autogenous bone to the bone substitute material Bio-Oss can produce a high-quality implant site. To improve vertical bone height, 13 sinus floor elevations were carried out in a total of 12 patients. Augmentation of the maxillary sinus floor was completed using a mixture of Bio-Oss and bone harvested intraorally from the mandibular symphysis, the retromolar space, or the tuberosity region. Following an average of 7.1 months of healing, 36 Brånemark System implants were placed. During this surgical intervention, 23 cylinder-shaped bone biopsies were taken from the augmented maxillary region using trephine burs. Histologic analysis of the bone biopsies revealed that the Bio-Oss granulate was well-integrated into the newly formed bone; 33.1% ( $\pm$  12.4%) of the substitute material surface was in direct contact with bone. Histomorphometric analysis of the samples revealed an average percentage proportion of bone of 18.9% ( $\pm$  6.4%). The bovine substitute material and soft tissue occupied, respectively, 29.6% ( $\pm$  8.9%) and 51.5% ( $\pm$  9.4%) of the measured surface. When the implants were uncovered after an average healing phase of 6 months, all 36 implants had become osseointegrated. The combination of osteoconductive Bio-Oss and osteoinductive autogenous bone thus proved to be a material suitable for application in sinus floor augmentation. (INT J ORAL MAXILLOFAC IMPLANTS 2001;16:23–33)*

**Key words:** bone substitutes, bone transplantation, endosseous dental implantation, osteoconduction, osteoinduction, sinus augmentation

Prosthetic treatment of the posterior maxilla with implants is often difficult because of proximity to the maxillary sinus and insufficient bone height. Early loss of the molars in the maxilla can result in the massive reduction of bone volume in both the vertical and

horizontal directions and thus unfavorable anatomic conditions for the surgical placement of endosseous implants.<sup>1</sup> Frequently, disappearance of the cancellous bone of the basal maxillary sinus precedes tooth loss, so that the root tips reaching into the maxillary sinus remain covered only by the Schneiderian membrane. Long-term lack of dentition in the maxilla can result in the reduction of the jawbone to paper thinness in the region of the alveolar recess.<sup>2</sup>

These irreversible resorptive processes following loss of the maxillary premolars and molars result from a number of factors. Increasing pneumatization of the maxillary sinus can result in an enlargement of the basal proportion of the maxillary sinus.<sup>3</sup> Also, increased osteoclastic activity in the maxillary sinus mucosa can produce resorption of the maxillary sinus floor. Atrophic processes can be accelerated by removable dentures.<sup>4</sup>

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However, in addition to the quantity of bone, a further factor requiring consideration in relation to the osseous anchoring of implants is bone quality. There is often no cortical bone in the posterior region of the maxilla, and frequently the cancellous bone is of only low density—factors that are disadvantageous for achieving primary stability with implant placement.<sup>5,6</sup> These facts explain the inferior long-term success rates for implants placed in the maxilla in comparison to the mandible.<sup>7,8</sup>

The technique of sinus floor elevation and subantral augmentation allows improvement of vertical bone height in the anatomically unfavorable posterior maxilla, while having the objective of placing implants, either at the time of operation or after a certain healing period. Tatum developed the surgical technique in the mid-1970s and reported it in 1986.<sup>9</sup> However, the first clinical results had been presented previously by Boyne and James.<sup>10</sup> Modifications of the technique were later presented by other authors.<sup>11,12</sup> The surgical technique involved in augmentation of the sinus floor consists of preparing a window in the region of the buccal maxillary sinus wall, medial-cranial rotation of the bone cover thus created, and simultaneous elevation of the maxillary sinus mucosa, followed by augmentation of the resulting cavity with autogenous bone and/or bone substitute material. Selection of appropriate augmentation material is one of the crucial factors in determining the outcome of sinus floor elevation.<sup>13</sup>

Augmentation of the sinus floor with autogenous bone from the iliac crest has demonstrated good success rates.<sup>10,14–18</sup> However, the second surgical operation in the region of the iliac crest greatly increases expenditure both in terms of time and financial cost. The harvesting of autotransplants from the iliac crest is frequently connected with morbidity and functional limitations from the patient's point of view.<sup>19</sup> Younger and Chapman reported a complication rate following this operation (in the form of infection, loss of blood, and pain) of 8.6%.<sup>20</sup> Donor sites in the maxillofacial region are also useful. Several authors have reported good success rates for sinus floor augmentation with autogenous bone harvested from the tuberosity region, the mandibular symphysis, or the retromolar space.<sup>11,21,22</sup> However, strict limits on the availability of this material present a disadvantage, in spite of the relatively low expenditure involved in transplant harvesting and reduced morbidity for patients. The quantity of bone harvested is generally insufficient for sinus floor augmentation. A number of authors have therefore advocated the use of transplant materials from other sources, such as

allografts,<sup>18,23</sup> hydroxyapatite from coral,<sup>24</sup> bovine bone,<sup>23,25</sup> or combinations of these materials,<sup>26–28</sup> for sinus floor elevation.

Histologic and histomorphometric analyses of bone biopsies removed from the augmentation region allow for evaluation of integration and resorption of the materials used, and thus evaluation of their suitability as bone substitute material. Osseous incorporation has been found in animal experiments with Bio-Oss (Geistlich Biomaterials, Wolhusen, Switzerland), which was used in the present study.<sup>23,29</sup> Individual particles of bovine apatite were found to be mostly surrounded by newly formed bone, thus having served as scaffolding in the formation of new bone. Use of bovine apatite in human bone has also produced good clinical results to date.<sup>30</sup> Since it was previously proven to be impossible to achieve osteoinduction with bone substitute material alone, the addition of autogenous material is recommended for improving the bone quality of the augmented region.<sup>26,31,32</sup>

The aim of the present study was to ascertain, through histologic and histomorphometric investigation of human specimens, whether it is possible to create a suitable implant site by adding autogenous bone to the xenogenic bone substitute material Bio-Oss.

## MATERIALS AND METHODS

The patient population comprised 12 individuals (9 female, 3 male) with an average age of 51.2 years (range, 32 to 65 years). Preoperative diagnosis with panoramic images and, in some cases, computed tomographic scans, showed that all patients had an insufficient residual bone supply (on average 1.88 mm) in the subantral maxillary region for immediate implant placement (Fig 1). Adequate implant sites were created by improving vertical bone height through sinus floor augmentation. Thirteen sinus floor elevations (bilaterally for 1 patient and unilaterally for the remaining 11) were completed for the 9 patients with partial dentition and the 3 completely edentulous patients. All operations were completed by the same surgeon between April 1996 and July 1998 (Table 1).

### Surgical Procedure

The sinus augmentations were completed according to the window rotation technique described by Boyne and James.<sup>10</sup> Patients were administered local anesthesia, and an incision was made in a slightly palatal position along the alveolar crest. Vertical releasing incisions were also made anteriorly and posteriorly.

**Fig 1** Preoperative panoramic radiograph. The vertical bone supply in the posterior region of the maxilla is insufficient for implant placement.



Next, a superiorly based mucoperiosteal flap was prepared and elevated, allowing an adequate view of the buccal sinus wall. Initially with a Lindemann bur and subsequently with a diamond-tipped bur, a bony window was formed; the site was cooled continuously with sterile saline (Fig 2a). The Schneiderian membrane was left intact. The fractured lateral sinus wall was elevated to the medial-cranial (Fig 2b). The cavity thus created was filled with the augmentation material (Fig 2c), which was Bio-Oss mixed with autogenous bone. The autotransplant was harvested intraoperatively from the mandibular symphysis, the retromolar space, or the tuberosity region (Table 1). Pieces of bone were harvested from each of the donor regions via trephined circular holes 8 mm in diameter (Fig 2d). Larger particles of bone were reduced to fine chips using a bone grinder and mixed with Bio-Oss. After application of the augmentation material, a resorbable Bio-Gide membrane (Geistlich Biomaterials) was used to cover the defect in the buccal maxillary sinus wall to prevent soft tissue from growing into the augmented region. The operated region was then sutured using non-resorbable 5.0 sutures (GTAM, W. L. Gore, Flagstaff, AZ).

In the postoperative phase, patients were protected from infection by administration of the antibiotic Augmentin (500 mg, 4 times daily) (Beecham-Wülfing, Neuss, Germany). To prevent swelling of the nasal and maxillary mucous membranes, Otrivin (CIBA-Geigy, Wehr, Germany) was prescribed. Patients were also given ibuprofen (400 mg, 3 times daily) as an analgesic and antiphlogistic (Klinge Pharma, Munich, Germany). They were instructed to rinse twice daily over a period of 2 weeks with a 0.2% chlorhexidine gluconate solution. Patients were also advised not to wear their removable prosthesis during the same period and not to blow their noses. Following a healing period of 2 weeks, the sutures were removed.

After an average of 7.1 months of healing (range, 6.0 to 9.5 months), a total of 36 Brånemark System implants (Nobel Biocare AB, Göteborg, Sweden) were placed in the posterior maxillae of the 12 patients (Figs 3a and 3b). During this surgery, 23 bone biopsies were removed from the augmentation material. This was completed using a trephine bur of 2.1-mm inner diameter and 3-mm outer diameter. Intraoral implants were placed according to standard procedure into the bone cavities thus created.

#### Preparation of Ground Specimens

For histologic and histomorphometric analyses of the bone biopsies, histologic sections were completed according to the standard sawing and grinding technique of Donath and Breuner.<sup>33</sup> The trephine burs were immersed in formalin for 48 hours, rinsed in running water overnight, and then dehydrated in serial concentrations of alcohol (70%, 80%, 96%, and 100%). The sections were embedded in the plastic K-Plast (Medim, Giessen, Germany). Two sections, each approximately 300  $\mu$ m thick, were cut parallel to the longitudinal axis of the trephine bur using a diamond band saw (Exakt, Norderstedt, Germany) (Fig 4). The cuts were then reduced by microgrinding and polishing to a thickness of 100  $\mu$ m and stained, first with toluidine blue and then with pyronine G. This allowed 2 histologic sections to be produced from each biopsy.

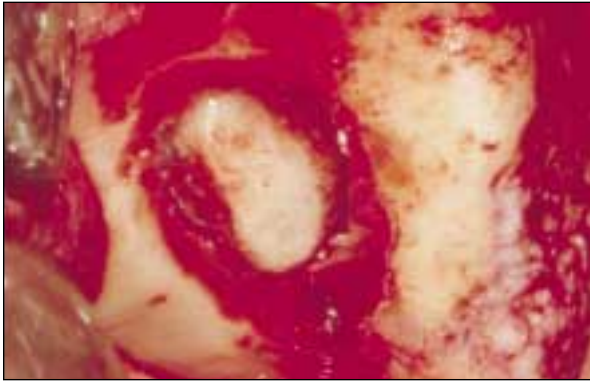
#### Histology

The newly formed bone could be unambiguously distinguished from bovine bone substitute material. Staining with toluidine blue and pyronine G caused the newly formed osseous structures and Bio-Oss to appear purple and bright purple-orange, respectively. The xenogenic bone substitute material exhibits empty osteocyte lacunae characterized by a lack of osteocyte nuclei and a washed-out or completely

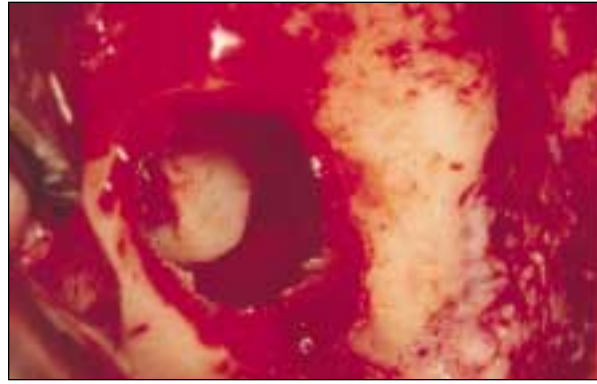
Table 1 Patient Population Data

Patient	Sex	Date of birth	Residual bone (mm)	Date of sinus augmentation	Donor site	Date of implant placement	Resting time (mo)	Quadrant	Location*	Length (mm)	Diameter (mm)	Implant type	Biopsy	Complications following augmentation
P.B.	M	1936	2.20	4/96	Symphysis	10/96	6.0	I	14	13.0	3.75	MkII	No	None
									15	15.0	3.75	MkII	Yes	
									16	15.0	3.75	MkII	No	
M.H.	F	1957	2.10	4/96	Symphysis	10/96	6.0	I	15	11.5	3.75	MkII	No	Suture dehiscence
									16	13.0	3.75	MkII	Yes	
									17	13.0	3.75	MkII	Yes	
									18	11.5	3.75	MkII	No	
B.S.	F	1964	2.80	8/96	Retromolar	2/97	6.0	II	26	13.0	3.75	Standard	No	None
									27	13.0	3.75	Standard	Yes	
G.N.	M	1938	2.72	10/96	Retromolar	4/97	6.0	I	15	15.0	3.75	MkII	No	None
									16	13.0	5.00	MkII	Yes	
									17	10.0	5.00	MkII	Yes	
S.F.	F	1953	1.30	4/97	Symphysis	2/98	9.5	I	15	15.0	3.75	MkII	No	None
									16	15.0	3.75	MkII	Yes	
									17	13.0	3.75	MkII	Yes	
S.C.	M	1947	1.92	6/97	Symphysis	3/98	9.0	I	15	13.0	3.75	MkII	No	None
									16	15.0	3.75	MkII	Yes	
									17	13.0	3.75	Standard	Yes	
B.V.	F	1944	1.00	6/97	Symphysis	3/98	9.0	I	16	10.0	5.00	MkII	Yes	None
									17	11.5	5.00	MkII	Yes	
K.R.	F	1947	2.20	6/97	Symphysis	1/98	7.5	I	15	13.0	3.75	MkII	No	None
									16	13.0	5.00	MkII	Yes	
									17	11.5	5.00	MkII	Yes	
G.P.	F	1945	2.08	7/97	Symphysis	2/98	6.5	I	15	13.0	3.75	MkII	No	Nosebleed
									16	13.0	3.75	MkII	Yes	
									17	8.5	5.00	MkII	Yes	
									25	13.0	3.75	MkII	No	
									26	13.0	3.75	MkII	Yes	
									27	13.0	5.00	MkII	No	
L.K.	F	1934	0.80	9/97	Tuberosity	3/98	6.5	I	15	15.0	3.75	MkII	No	None
									16	13.0	4.00	Standard	Yes	
									17	13.0	4.00	Standard	Yes	
K.G.	F	1948	1.10	9/97	Tuberosity	4/98	7.5	II	26	13.0	5.00	MkII	Yes	Nosebleed
									27	13.0	5.00	MkII	Yes	
A.P.	F	1932	3.10	1/98	Tuberosity	7/98	6.5	I	16	13.0	5.00	MkII	Yes	None
									17	13.0	5.00	MkII	Yes	

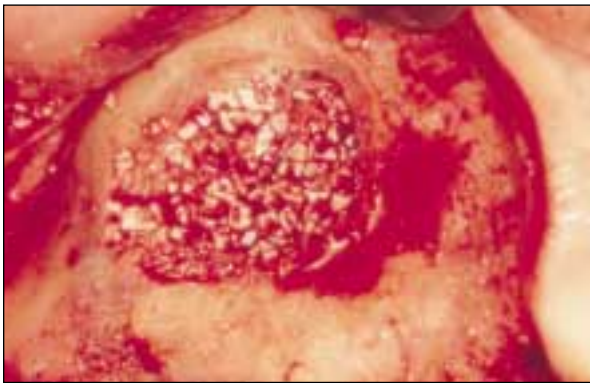
\* Tooth numbers: 14 = R, first premolar; 15 = R, second premolar; 16 = R, first molar; 17 = R, second molar; 18 = R, third molar; 19 = R, second premolar; 20 = L, second premolar; 21 = L, first molar; 22 = L, second molar; 23 = L, third molar; 24 = L, second premolar; 25 = L, second premolar; 26 = L, second premolar; 27 = L, second molar.



**Fig 2a** The lateral maxillary sinus wall with prepared bone cover.



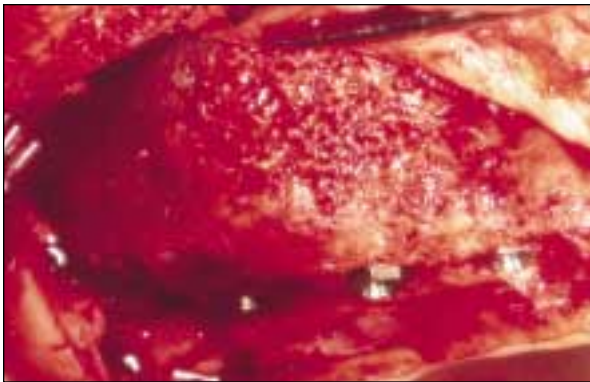
**Fig 2b** The lateral maxillary sinus wall is rotated to the medial-cranial, and the Schneiderian membrane is lifted without perforation.



**Fig 2c** The xenograft/autograft mixture is applied to the maxillary sinus floor.



**Fig 2d** Donor areas are outlined for the autogenous bone transplant. Bone is removed from the chin region using a trephine bur.



**Fig 3a** Clinical status 7.5 months after augmentation of the maxillary sinus floor in the right quadrant. During implant placement, test biopsies are harvested from the augmented region.



**Fig 3b** Panoramic image following prosthetic treatment of the implants with metal-ceramic interlocking implant crowns.

missing lamella-type layer. In contrast, osteocytes sending out branched protoplasmic processes into the small bone canals are easily recognizable in the flattened lacunae osseae of the natural living bone tissue. Also, the bovine granules are short, thick, and sharp-edged, whereas the natural bone lamellae are long and the boundaries relatively unclear (Fig 5a).

The specimens were viewed under polarized light so that the Bio-Oss and newly formed bone could be clearly distinguished. It was thus possible to highlight the difference between implanted and newly formed bone via visualization of the birefringent fibers in the bone (Fig 5b).

### Histomorphometric Analyses

A semiautomatic measurement technique was selected for histomorphometric investigation of the fine-ground specimens. The operating system applied was Windows for Workgroups 3.11 (Microsoft, Munich, Germany) with its graphic user interface. Image processing was performed with the analysis program KS 300 (Zeiss, Jena, Germany). The hardware required for comprehensive



**Fig 4** Longitudinal section through the trephine bur with removed sample material.



**Fig 5a** Histologic specimen of the augmentation material (original magnification  $\times 100$ ). The individual Bio-Oss particles are fully integrated into the newly formed bone.

measurement of the sections was Prog/Res/3008 (Kontron Elektronik, Eching, Germany) and consisted of the light microscope Axioskop (Zeiss, Jena, Germany), a videocamera, a video mixer, and an IBM-compatible computer. The visual field of the light microscope was recorded by the videocamera at a magnification of 100, and the field was projected onto the computer screen using the video mixer with the computer graphics of the image processing program KS 300. Movement of the section, and thus adjustment of the measurement field (which matched the visual field of the microscope), was possible via 2 screws on the stage of the microscope. Selection of measurement fields was performed by visually monitoring the microscopic image on screen. The cursor was used to move over the surface of the Bio-Oss particles and newly formed bone. Special macroprogramming achieved automatic calculation of the substance of the surface being moved over via pixel counting (Fig 6).

Since the visual field remained at a defined size of 0.5 mm<sup>2</sup>, the software was able to calculate both the proportions of substitute material and newly formed bone and, through differential calculus, the proportion of soft tissue over the entire measured surface. During a second measurement phase, the cursor was used to mark the size of the Bio-Oss particles and the regions over which they were covered by newly formed bone. It was then possible to calculate the percentage of Bio-Oss granulate that was in direct contact with bone.

Two sections were obtained from each biopsy. The sections were of different width according to the cross section of the trephine. This meant that in each pair of ground specimens, one contained a single-row measuring field, while the other contained a double-row measuring field. During surgery, the trephine bur was sunk to different lengths according



**Fig 5b** The same specimen viewed under polarized light. The collagen fibers present in the newly formed bone make it appear much lighter in color than the bovine bone replacement material.

to the depth of the implants subsequently placed; this resulted in a variation in the total number of fields to be measured for each histologic preparation, ie, between 4 and 28. Regions in which residual bone was also cut by the trephine bur were excluded from histomorphometric analysis, so as to avoid bias in the measurement values.

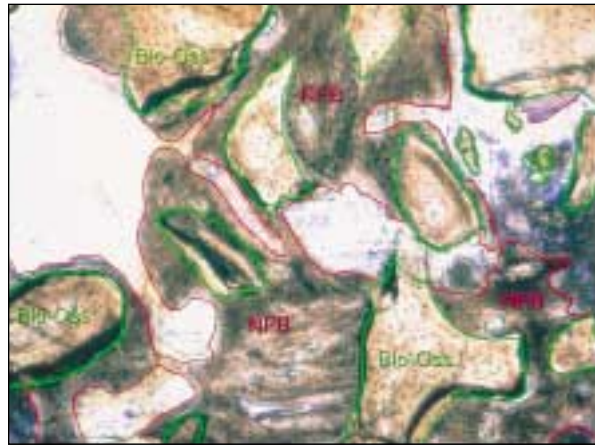
## RESULTS

### Clinical Observations

The healing period following maxillary sinus augmentation was completed for nearly all patients without complications. Two patients showed a postoperative tendency for nosebleed that lasted 3 days, and another patient presented with dehiscence of a suture. No clinical symptoms indicating maxillary sinusitis occurred in any of the 13 sinus augmentations. The intraoral implants placed during the second operation healed well. After an average of 6 months, the implants were surgically uncovered. All 36 implants were found to be osseointegrated, resulting in a 100% implant survival rate at this time. Prosthetic treatment of the patients consisted of either an implant-supported removable prosthesis or an entirely implant-supported superstructure. Long-term studies relating to prosthetic loading are pending.

### Histologic Observations

Even after a resting time for Bio-Oss of up to 9.5 months in some cases, individual particles of bone substitute material were still clearly identifiable. Histologic analysis showed that the newly formed bone was in direct contact with the Bio-Oss particles. The xenograft was not only invaginated into the newly formed bone, but individual granules had also become interconnected through trabecula formation. The bone was mainly woven, with more mature lamellar bone occurring only in isolated instances. In some sections, it was possible to demonstrate vascular and osseous apposition of the bovine apatite. The cancellous trabeculae of the xenogenic material served as a scaffold for the newly forming bone, a process described in the literature as *osteoconduction*. The soft tissue located between the trabeculae and the xenogenic substitute material contained connective tissue composed of various forms of fibroblasts, collagenous fibers, and blood vessels and showed no signs of inflammation. On light microscopic examination, neither resorption lacunae nor active osteoclasts were found. At the same time, penetrative ingrowth of the bone indicated that the xenograft was being slowly substituted.



**Fig 6** Computer image of the histologic slides. In the histomorphometric evaluation, the Bio-Oss particles are marked green, while the newly formed bone is marked red.

### Histomorphometric Observations

Table 2 and Fig 7 show the results of histomorphometric measurements. The average percentage proportion of newly formed bone was  $18.9\% \pm 6.4\%$ , in which individual measurement values ranged from 12.9% to 36.1%. The porous substitute material of bovine origin occupied an average of  $29.6\% \pm 8.9\%$  of the measured surface. With a value of  $51.5\% \pm 9.3\%$ , the average percentage proportion of soft tissue accounted for more than half of the augmented area. Figure 8 shows the mean values of the histomorphometric results for individual patients and regions. Even in the same patient, some considerable differences were noticed in relation to the percentage proportion of bone in adjoining regions. In spite of the waiting period of 6.0 to 9.5 months prior to removal of the bone biopsies, the duration of placement was not identifiable as a factor affecting the percentage proportion of bone. Thus, the individual healing response in individual patients, rather than the length of time that the substitute material remained in place, was seen to have an effect on the integration of the xenograft.

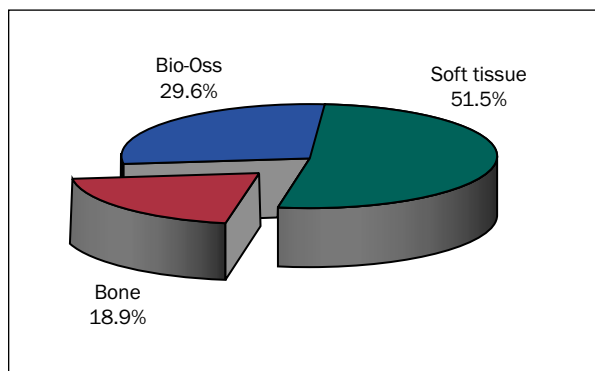
Bone growth around Bio-Oss, as seen in the analyzed sections, showed considerable differences regardless of patient age or sex. Values ranged from 9.8% to 53.2% and resulted in an average invagination of the granulate of  $33.1\% \pm 12.4\%$ . Once more, the duration of xenograft placement could not be identified as a factor affecting the extent to which bone grew around it. Integration of the bone substitute seemed to be influenced more by the specific healing response of the individual patient.

**Table 2** Histomorphometry (Mean  $\pm$  SD, in Percentages) of Sinus Biopsies Following Grafting with Bio-Oss and Autogenous Bone

Patient	Location	Bone	Bio-Oss	Soft tissue
P.B.	15	16.3 $\pm$ 0.6	23.3 $\pm$ 3.6	60.4 $\pm$ 3.0
M.H.	16	15.9 $\pm$ 1.4	28.1 $\pm$ 8.0	55.9 $\pm$ 9.4
	17	13.3 $\pm$ 0.9	32.1 $\pm$ 7.7	54.7 $\pm$ 6.8
B.S.	27	16.4 $\pm$ 1.4	30.4 $\pm$ 3.4	53.1 $\pm$ 2.0
G.N.	16	14.7 $\pm$ 2.6	29.3 $\pm$ 5.5	56.1 $\pm$ 2.9
	17	15.9 $\pm$ 0.5	23.0 $\pm$ 0.9	61.1 $\pm$ 1.4
S.F.	16	15.1 $\pm$ 1.1	38.4 $\pm$ 9.7	46.5 $\pm$ 9.0
	17	18.6 $\pm$ 0.1	30.0 $\pm$ 4.8	51.5 $\pm$ 4.8
S.C.	16	20.9 $\pm$ 4.4	26.8 $\pm$ 1.3	52.2 $\pm$ 3.1
	17	18.9 $\pm$ 2.7	42.9 $\pm$ 5.7	38.2 $\pm$ 8.4
B.V.	16	12.9 $\pm$ 4.1	31.7 $\pm$ 1.5	55.4 $\pm$ 5.6
	17	22.8 $\pm$ 8.1	33.8 $\pm$ 4.4	43.4 $\pm$ 3.7
K.R.	16	12.9 $\pm$ 1.3	46.1 $\pm$ 9.7	41.0 $\pm$ 11.0
	17	13.8 $\pm$ 2.4	25.9 $\pm$ 3.0	60.4 $\pm$ 0.6
G.P.	16	13.8 $\pm$ 0.6	30.4 $\pm$ 8.0	55.9 $\pm$ 7.4
	17	23.1 $\pm$ 3.7	43.8 $\pm$ 8.1	33.2 $\pm$ 4.5
	26	17.9 $\pm$ 3.1	28.1 $\pm$ 6.5	54.0 $\pm$ 3.4
L.K.	16	17.1 $\pm$ 1.4	30.5 $\pm$ 8.4	52.4 $\pm$ 9.8
	17	18.4 $\pm$ 2.3	14.0 $\pm$ 2.8	67.6 $\pm$ 0.5
K.G.	26	31.9 $\pm$ 6.3	22.8 $\pm$ 6.7	45.3 $\pm$ 13.1
	27	36.1 $\pm$ 1.8	22.0 $\pm$ 3.6	41.9 $\pm$ 1.7
A.P.	16	21.6 $\pm$ 4.9	30.0 $\pm$ 2.7	48.4 $\pm$ 7.5
	17	27.1 $\pm$ 0.7	18.3 $\pm$ 9.1	54.7 $\pm$ 9.0
Overall mean $\pm$ SD		18.9 $\pm$ 6.4	29.6 $\pm$ 8.9	51.5 $\pm$ 9.4

The mean values shown for standard deviations relate to the total number of histomorphometric measurements per measurement field carried out.

\*Tooth numbers: 15 = R. second premolar; 16 = R. first molar; 17 = R. second molar; 26 = L. first molar; 27 = L. second molar.



**Fig 7** Histomorphometric findings (mean values) after grafting with Bio-Oss and autogenous bone.

## DISCUSSION

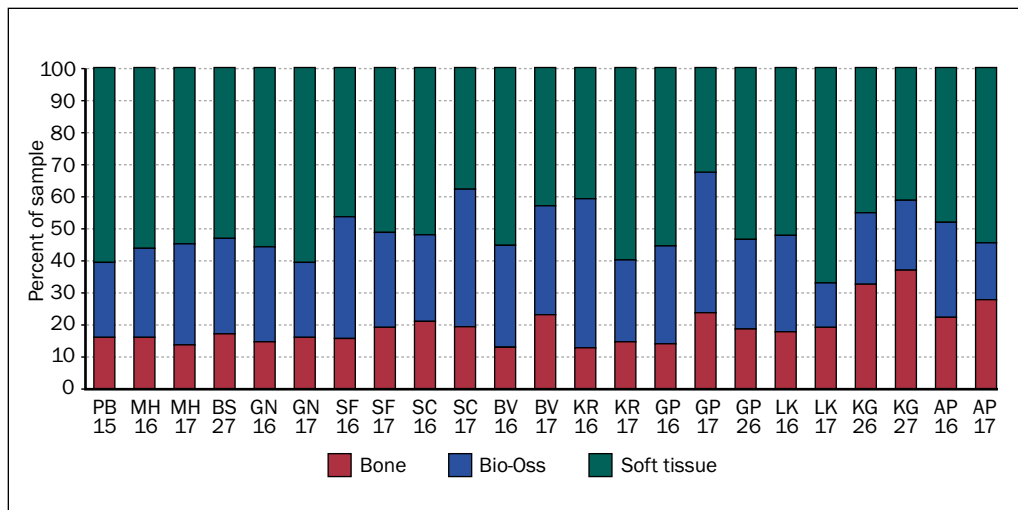
Because of its osteogenic potential, the use of autogenous bone in sinus floor augmentation is regarded as the most reliable method for obtaining high-quality potential implant sites. Formation of new bone is

activated by induction materials, such as bone morphogenetic protein and special growth factors that promote differentiation of the osteoprogenitor cells into osteoblasts and/or chondroblasts.<sup>26,34</sup>

The histomorphometrically obtained results for bone apposition of bovine apatite in animal models are frequently greater than the values arrived at in the present study. However, the applicability of animal experiments to humans is extremely problematic. For example, the bone reconstruction rate for rodents is much higher than that of humans. Physiologic remodeling in rabbits is approximately 3 times faster than in humans.<sup>35</sup> Use of bovine bone substitute material in humans must therefore be expected to result in a lower proportion of new bone formation.

In a clinical study, Wheeler et al<sup>36</sup> used Interpore 200 (Interpore International, Irvine, CA) and Bio-Oss for the sinus augmentation procedure. After a healing period of 4 to 36 months, bone biopsies were removed and histomorphometrically measured. The use of hydroxyapatite alone resulted in a bone density of 16.38%.<sup>36</sup> Valentini et al completed





**Fig 8** Histomorphometric results for individual patients and individual regions. Tooth numbers: 15 = R. second premolar; 16 = R. first molar; 17 = R. second molar; 26 = L. first molar; 27 = L. second molar.

sinus augmentation for 1 patient using Bio-Oss.<sup>37</sup> This was followed by a histomorphometric comparison of the proportion of bone in the residual bone with the proportion of new bone formation in the augmented region. In the residual bone, the bone density measured was 27%, and in the augmentation material new bone formation of 28% was achieved, with a density of bone substitute material of 28%. The demand for space of the bone substitute material would thus seem to limit itself to the bone marrow region.<sup>37</sup> Camelo et al investigated the clinical application of Bio-Oss in periodontal defects.<sup>38</sup> After a resting time of the bone substitute material of 6 to 9 months, biopsies were removed from the relevant regions for histomorphometric analysis. In a total of 4 patients, the percentage values for new bone formation varied between 5.2% and 31.6%. The healing response in individual patients seemed to be a crucial factor.

The average percentage proportion of newly formed bone obtained by the authors ( $18.9\% \pm 6.4\%$ ) after an average bovine apatite resting time of 7.1 months is comparable with the above-cited investigations. The values measured in the present study also showed a relatively large range: the lowest value was 12.9%, and the highest was 36.1%.

The present study failed to confirm the increase in percentage bone proportion obtained by Haas et al following sinus augmentation with bovine hydroxyapatite in sheep (27.4% bone after 12 weeks resting time versus 34.7% bone after 26 weeks).<sup>25</sup> Although sampling was completed after an augmentation material resting time of between 5.5 and 10.5

months, an increase in the percentage proportion of bone over time was not seen. Accordingly, bony integration of Bio-Oss is mainly influenced by the healing response of the individual patient and is less dependent on the resting time of the augmentation material.

In the literature, resorption of bovine bone substitute material has been the subject of controversy. Resorption of Bio-Oss has been described for animal experiments in the rabbit and dog.<sup>39,40</sup> Schlickewei and Paul described resorption of Bio-Oss as physiologic remodeling, requiring (as expected) a time interval of 1 to 5 years in the case of human bone and 6 to 12 months in the case of rodents.<sup>29</sup> In humans, radiographic examination has been able to identify the presence of Bio-Oss granules even after a resting time of up to 7 years<sup>30</sup> and histologically 44 months after augmentation of the maxillary alveolar ridge.<sup>41</sup> This leads the authors to doubt the resorbability of the material. Osteoclastic activity seemed to affect the appositionally developed bone of the patient, which was itself oriented to the bovine pattern, rather than the bovine structure of the substitute material.<sup>30</sup>

Histologically, the present study was unable to prove the presence of resorption lacunae or osteoclasts. However, penetrative inward bone growth points to biologic degradation of the bone substitute material. At the outset, Bio-Oss apparently accounts for 25 to 30% of the space available in the defect.<sup>42</sup> Given that in the present study the average density of the substitute material was 29.6%, a high level of osteoclastic activity cannot be hypothesized.

Slow resorption as physiologic remodeling when Bio-Oss is used in sinus floor augmentation appears appropriate, because rapidly progressing degradation would endanger the stability of the implant site.

The histologically observed integration of the Bio-Oss granulate, as well as the implant osseointegration confirmed at uncovering, suggests that a combination of Bio-Oss and autogenous bone can be useful as augmentation material. Long-term studies under prosthetic loading will be required to confirm whether the maxillary implant success rate of 84.9% observed by Albrektsson et al after an observation period of 5 to 7 years can be achieved for implants placed in augmented regions.<sup>7</sup>

## CONCLUSIONS

The present study demonstrated that sinus floor augmentation with a blend of intraorally harvested bone and xenogenic Bio-Oss as augmentation material can be regarded as an appropriate method of treatment for achieving an increase in subantral bone supply for implant placement. The histologically observed integration of bovine apatite, together with the 100% survival rate at the time of implant uncovering, supports clinical application for patients.

The quality of the implant site may be enhanced by the addition of autogenous bone. By providing osteoblasts, intraorally harvested bone can contribute to new bone formation. Osteoinductive properties of the autograft related to bone morphogenetic proteins simultaneously achieve differentiation of mesenchymal cells into bone-forming cells. The combination of osteoinductive autograft and osteoconductive xenograft thus appears promising. Long-term results under prosthetic loading will provide information about whether maxillary sinus augmentation using Bio-Oss and bone harvested from the patient can ensure a suitable implant site over the long term.

## REFERENCES

1. Watzek G, Ulm CW, Haas R. Anatomic and physiologic fundamentals of sinus floor augmentation. In: Jensen OT (ed). *The Sinus Bone Graft*. Chicago: Quintessence, 1999:31–47.
2. Plenck H, Tschabitscher M. Entwicklung, Makro- und Mikromorphologie der Kieferhöhle. In: Watzek G, Matejka M (eds). *Erkrankungen der Kieferhöhle*. New York: Springer, 1985:1–12.
3. Wehrbein H, Diedrich P. Die fortschreitende Pneumatisation der basalen Kieferhöhle nach Extraktion und Lückenschluß. *Fortschr Kieferorthop* 1992;53:77–83.

4. Chanavaz M. Maxillary sinus: Anatomy, physiology, surgery, and bone grafting related to implantology—Eleven years of surgical experience (1979–1990). *J Oral Implantol* 1990;16:199–209.
5. Misch CE. Density of bone: Effect on treatment plans, surgical approach, healing, and progressive bone loading. *Int J Oral Implantol* 1990;6:23–31.
6. Spiekermann H. *Implantology*. New York: Thieme, 1995.
7. Albrektsson T, Dahl E, Enbom L, Engvall S, Engquist B, Eriksson AR, et al. Osseointegrated oral implants: A Swedish multicenter study of 8139 consecutively inserted Nobel-pharma implants. *J Periodontol* 1988;59:287–296.
8. Adell R, Eriksson B, Lekholm U, Brånemark P-I, Jemt T. A long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. *Int J Oral Maxillofac Implants* 1990;5:347–359.
9. Tatum H. Maxillary and sinus implant reconstructions. *Dent Clin North Am* 1986;30:207–229.
10. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg* 1980;38:613–616.
11. Wood RM, Moore DL. Grafting of the maxillary sinus with intraorally harvested autogenous bone prior to implant placement. *Int J Oral Maxillofac Implants* 1988;3:209–214.
12. Smiler DG, Johnson PW, Lozada JL, Misch C, Rosenlicht JL, Tatum OH, Wagner J. Sinus lift grafts and endosseous implants: Treatment of the atrophic posterior maxilla. *Dent Clin North Am* 1992;36:151–186.
13. Jensen OT, Shulman LB, Block MS, Iacono VJ. Report of the sinus consensus conference of 1996. *Int J Oral Maxillofac Implants* 1998;13(suppl):S11–S32.
14. Kent JN, Block MS. Simultaneous maxillary sinus floor bone grafting and placement of hydroxylapatite-coated implants. *J Oral Maxillofac Surg* 1989;47:238–242.
15. Jensen J, Krantz-Simonsen EK, Sindet-Pedersen S. Reconstruction of the severely resorbed maxilla with bone grafting and osseointegrated implants: A preliminary report. *J Oral Maxillofac Surg* 1990;48:27–32.
16. Raghoobar GM, Brouwer TJ, Reintsema H, Van Oort RP. Augmentation of the maxillary sinus floor with autogenous bone for the placement of endosseous implants: A preliminary report. *J Oral Maxillofac Surg* 1993;51:1198–1203.
17. Keller EE, Eckert SE, Tolman DE. Maxillary antral and nasal one-stage inlay composite bone graft: Preliminary report on 30 recipient sites. *J Oral Maxillofac Surg* 1994;52:438–447.
18. Nishibori M, Betts NJ, Salama H, Listgarten MA. Short-term healing of autogenous and allogenic bone grafts after sinus augmentation: A report of 2 cases. *J Periodontol* 1994;65:958–966.
19. Gerngross H, Burri C, Kinzl L, Merk J, Müller GW. Komplikationen an der Entnahmestelle autologer Spongiosatransplantate. *Aktuel Traumatol* 1982;12:146–152.
20. Younger EM, Chapman MW. Morbidity at bone graft donor sites. *J Orthop Trauma* 1989;3:192–195.
21. Hirsch JM, Ericsson I. Maxillary sinus augmentation using mandibular bone grafts and simultaneous installation of implants. A surgical technique. *Clin Oral Implants Res* 1991;2:91–96.
22. Lundgren S, Moy P, Johansson C, Nilsson H. Augmentation of the maxillary sinus floor with particulated mandible: A histologic and histomorphometric study. *Int J Oral Maxillofac Implants* 1996;11:760–766.

23. Wetzel AC, Stich H, Caffesse RG. Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. *Clin Oral Implants Res* 1995;6:155-163.
24. Smiler DG, Holmes RE. Sinus lift procedure using porous hydroxyapatite: A preliminary clinical report. *J Oral Implants Res* 1987;13:239-253.
25. Haas R, Donath K, Födinger M, Watzek G. Bovine hydroxyapatite for maxillary sinus grafting: Comparative histomorphometric findings in sheep. *Clin Oral Implants Res* 1998;9:107-116.
26. Moy PK, Lundgren S, Holmes RE. Maxillary sinus augmentation: Histomorphometric analysis of graft materials for maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 1993;51:857-862.
27. Small SA, Zinner IA, Panno FV, Shapiro HJ, Stein JI. Augmenting the maxillary sinus for implants: Report of 27 patients. *Int J Oral Maxillofac Implants* 1993;8:523-528.
28. Valentini P, Abensur D. Sinusbodenelevation zur Implantation mit demineralisiertem, gefriergetrockneten Knochen und Rinderknochen (Bio-Oss): Eine klinische Studie mit 20 Patienten. *Int J Parodont Rest Zahnheilkd* 1997;17:217-225.
29. Schlickewei W, Paul C. Experimentelle Untersuchung zum Knochenersatz mit bovinem Apatit. In: Huggler AH, Kuner EH (eds). *Aktueller Stand beim Knochenersatz. Hefte zur Unfallheilkunde*. Berlin: Springer, 1991:59-69.
30. Schlegel AK. Bio-Oss bone replacement materials. Long-term results with Bio-Oss bone replacement material [in German]. *Schweiz Monatsschrift Zahnmedizin* 1996;106:141-149.
31. Misch CE, Dietsch F. Bone-grafting materials in implant dentistry. *Implant Dent* 1993;2:158-167.
32. Block MS, Kent JN. Sinus augmentation for dental implants: The use of autogenous bone. *J Oral Maxillofac Surg* 1997;55:1281-1286.
33. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. *J Oral Pathol* 1982;11:318-326.
34. Garg AK. Grafting materials in repair and restoration. In: Lynch SE, Genco RJ, Marx RE (eds). *Tissue Engineering: Applications in Maxillofacial Surgery and Prosthodontics*. Chicago: Quintessence, 1999:83-101.
35. Roberts WE, Turley PK, Brezniak N, Fielder PJ. Bone physiology and metabolism. *J Calif Dent Assoc* 1987;15:54-61.
36. Wheeler SL, Holmes RE, Calhoun CJ. Six-year clinical and histologic study of sinus-lift grafts. *Int J Oral Maxillofac Implants* 1996;11:26-34.
37. Valentini P, Abensur D, Densari D, Graziani JN, Hämmerle C. Histological evaluation of Bio-Oss in a 2-stage sinus floor elevation and implantation procedure: A human case report. *Clin Oral Implants Res* 1998;9:59-64.
38. Camelo M, Nevins ML, Schenk RK, Simion M, Rasperini G, Lynch SE, Nevins M. Clinical, radiographic, and histologic evaluation of human periodontal defects treated with Bio-Oss and Bio-Gide. *Int J Periodontics Restorative Dent* 1998;18:321-331.
39. Klinge B, Alberius P, Isaksson S, Jönsson J. Osseous response to implanted natural bone mineral and synthetic hydroxylapatite ceramic in the repair of experimental skull bone defects. *J Oral Maxillofac Surg* 1992;50:241-249.
40. Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss. An experimental study in the dog. *Clin Oral Implants Res* 1997;8:117-124.
41. Skoglund A, Hising P, Young C. A clinical and histologic examination in humans of the osseous response to implanted natural bone mineral. *Int J Oral Maxillofac Implants* 1997;12:194-199.
42. Peetz M. Characterization of xenogeneic bone material. In: Boyne PJ (ed). *Osseous Reconstruction of the Maxilla and the Mandible: Surgical Techniques Using Titanium Mesh and Bone Mineral*. Chicago: Quintessence, 1997:87-100.