Clinical, Histological, and Histomorphometrical Analysis of Maxillary Sinus Augmentation Using Inorganic Bovine in Humans: Preliminary Results

Joseph Bassil, PhD[1]*
Nada Naaman, PhD[2]
Raed Lattouf, DDS, MSc[3]
Cynthia Kassis, DDS[4]
Sylvie Changotade, PhD[5]
Brigitte Baroukh, PhD[6]
Karim Senni, PhD, DSc[7]
Gaston Godeau, PhD, DSc[8]

The aim of the present study was to evaluate bone formation after maxillary sinus augmentation using bovine bone substitute material Bio-Oss alone by means of clinical, histological, and histomorphometrical examination of human biopsies. Deproteinized bovine bone (DPBB, Bio-Oss) was used to fill cavities after elevation of the sinus mucosa following major sinus pneumatization. Twenty patients with edentulous posterior maxillae were treated with 20 sinus augmentation procedures using a 2-stage technique. Residual lateral maxillary bone height was less than 3 mm. Forty-nine Straumann endosseous implants were used to complete the implant-prosthetic rehabilitation. Forty cylinder-shaped bone biopsies were taken from the augmented maxillary region 8 months after grafting during the second-stage surgery before implant placement. All implants were loaded 3 months after insertion, and no failures were recorded. Histomorphometrical analysis showed an average percentage of newly formed bone of 17.6% (±2.8%) and a proportion of residual bone substitute material of 29.9% (±4.9%) of the total biopsy area. Intimate contact between newly formed bone and Bio-Oss was detected along 28.2% (±6.8%) of the particle surfaces. The results also showed that in all cases, the DPBB granules had been interconnected by bridges of vital newly formed bone. Inorganic bovine bone appears to be biocompatible and osteoconductive, and it can be used with success as a bone substitute in maxillary sinus augmentation procedures.

Key Words: histomorphometrical analysis, inorganic bovine bone, bone graft

[1] Department of Oral Surgery, Faculty of Dental Medicine, St Joseph University, Beirut, Lebanon.
[2] Department of Periodontology, Faculty of Dental Medicine, St Joseph University, Beirut, Lebanon.
[3] Department of Oral Surgery, St Joseph University, Beirut, Lebanon.
[4] Department of Restorative and Esthetic Dentistry, Faculty of Dental Medicine, St Joseph University, Beirut, Lebanon.
[5] Laboratoire de Biomatériaux et Polymères de Spécialité, Université Paris, Bobigny, France.
[6] Laboratoire sur la Réparation et les Remodelages oro-faciaux, Faculté de Chirurgie Dentaire, Université Paris, Montrouge, France.
[7] Innovation Department, Seadev-Fermensys, Plouzané, France, and Laboratoire de Biomatériaux et Polymères de Spécialité, Université Paris, Bobigny France.
[8] Faculté de Chirurgie Dentaire, Université Paris, Montrouge, France.
* *Corresponding author,* e-mail: joe.bassil@yahoo.fr
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INTRODUCTION

The placement of implants in highly atrophic maxillae continues to be a major challenge in implant dentistry. One of the preferred options to resolve this problem is sinus floor elevation, which involves lifting the sinus floor and packing the space created with grafting material to provide adequate support for implants. From the various materials available for sinus grafting, autogenous bone is considered the gold standard because of its high biocompatibility, in addition to its osteoconductive, osteoinductive, and osteogenic potential.[1,2]

However, the collection of autogenous bone requires an extra surgical site for bone harvesting, which increases the risks of morbidity and discomfort, particularly when bone is harvested from an extraoral site.[3,4]

Bone substitutes are available in unlimited amounts and can be used alone or in combination with autogenous bone. Various bone grafting materials have been used in sinus augmentation procedures, including freeze-dried bone allografts,[5] xenografts,[6] and hydroxyapatite.[7] Among bone substitutes, inorganic bovine bone has been recommended by several authors.[8] Bio-Oss (Geistlich Biomaterials, Wolhusen, Switzerland) is a bone substitute that has been used and evaluated for bone augmentation purposes. The safety of this xenograft has been questioned with respect to transmission of bovine spongiform encephalopathy.[9] Schwartz and colleagues[10] and Taylor and colleagues[11] found that some trace of protein may remain. Nevertheless, the complete absence of protein was demonstrated by Benke and colleagues.[12] Although histological studies of specimens from animals have shown that Bio-Oss has bone-conductive properties,[13,14] questions remain as to whether there is an advantage to mixing bone substitutes like Bio-Oss with autogenous bone over using Bio-Oss alone. In this study on a maxillary sinus floor augmentation procedure, the authors have used bovine hydroxyapatite alone. The advantage of using bone substitutes alone before implant surgery is apparent as no donor site for harvesting autogenous bone is necessary. The purpose of this study was to investigate human biopsies histologically and histomorphometrically when Bio-Oss was used alone in a surgical practice setting for sinus lift procedures.

Materials and Methods

Patients

The study reports on 20 grafted sinuses in 20 patients (10 women, 10 men) with a mean age of 57 years (range, 40–74 years) who had to undergo sinus floor elevation because of lack of bone for the placement of endosseous implants.

The inclusion criteria were that less than 3 mm of alveolar bone remained in the floor of the (average, 1.9 mm). The preoperative planning consisted of clinical examination of the maxilla and radiographic assessment with orthopantomography and computerized tomography scan (Figure 1). Of the 20 patients, 5 smoked more than 10 cigarettes/day; therefore, smoking did not represent an exclusion criterion in the present investigation. All patients were in general good health with no specific diseases, and none of them were on chronic drug therapy. Preoperatively, they were informed about the surgical procedures, were asked for their full cooperation during treatment, and signed a written informed consent. The study was approved by the ethical committee of St Joseph University in Beirut, Lebanon.

Surgical technique

In all 20 maxillary sinus floor elevations, the same method of surgical approach was used under local anesthesia as described by other authors.[15–17] One hour before the surgical procedure, all patients received 1 g of amoxicillin. Immediately before the procedure, the surgical area was rinsed for 2 minutes with a 0.2% chlorhexidine solution. An anesthetic agent (articaine 4% with 1/100 000 adrenaline) was infiltrated locally.

The incision was situated at the top of the alveolar crest or slightly palatally. A vertical releasing incision was placed in the canine area to facilitate flap elevation. A mucoperiosteal flap was elevated, exposing the lateral wall of the sinus. A bony window was outlined with the ultrasonic device (Esacrom, Serraglio, Italy) using the 2 mm round tips without perforating the Schneiderian membrane.

Once mobility of the window was obtained, the sinus membrane was elevated, starting from the inferior border of the osteotomy site. The lateral window was pushed inward and upward, creating a new horizontal ceiling.

The Bio-Oss particles, ranging from 0.25 to 1.00 mm, were hydrated with a saline solution and gently packed into the sinus until they filled the entire cavity (Figure 2). Absorbable membrane was not used on the vestibular sinus wall as the periosteum remained intact. Interrupted 4-0 silk sutures were used to close a tension-free flap.

Postoperatively, the patients were given amoxicillin (1 g twice daily for 6 days), a nonsteroidal anti-inflammatory drug as needed, and a nasal decongestant twice daily for 10 days. Sutures were removed 10 days after surgery. Patients were instructed to rinse twice daily over a period of 2 weeks using a 0.2% chlorhexidine gluconate solution.

Table

Histomorphometrical results (%) of sinus biopsies after grafting with Bio-Oss

Patient	Age (Years)	Gender	New Bone	Bio-Oss	Soft Tissue	Contact Surface
C.K.	43	Male	20.9 ± 6.7	25.6 ± 2.9	53.5 ± 3.4	34.4 ± 7.5
M.B.	73	Female	15.7 ± 0.9	34.4 ± 3.2	49.9 ± 8.7	24.2 ± 4.6
E.K.	64	Female	17.6 ± 2.3	27.3 ± 6.9	55.1 ± 2.3	23.7 ± 8.2
F.H.	44	Female	20.4 ± 1.2	31.8 ± 7.4	47.8 ± 0.5	36.3 ± 9.0
J.E.	48	Male	23.3 ± 3.4	17.6 ± 1.5	59.1 ± 8.2	32.9 ± 7.1
A.S.	69	Male	14.5 ± 1.5	32.2 ± 4.4	53.3 ± 3.7	21.3 ± 6.8
H.B.	74	Male	14.2 ± 1.7	33.1 ± 3.7	52.7 ± 5.6	22.0 ± 5.9
V.F.	47	Female	19.9 ± 4.9	20.5 ± 1.1	59.6 ± 7.2	30.9 ± 8.3
S.M.	58	Male	18.5 ± 6.2	28.7 ± 1.4	52.8 ± 5.9	32.2 ± 7.4
S.C.	54	Male	18.9 ± 3.6	25.4 ± 2.0	55.7 ± 6.5	24.5 ± 6.9
H.F.	49	Female	17.2 ± 0.6	30.9 ± 5.9	51.9 ± 2.7	27.9 ± 5.6
W.A.	74	Female	14.7 ± 1.1	41.6 ± 9.2	43.7 ± 1.2	24.2 ± 4.8
J.H.	75	Male	15.5 ± 1.4	39.7 ± 8.7	44.8 ± 2.0	26.6 ± 3.9
N.M.	56	Female	16.3 ± 0.9	27.2 ± 8.3	56.5 ± 3.3	25.4 ± 7.7
Y.A.	40	Female	19.5 ± 8.0	21.5 ± 2.6	59 ± 6.1	33.1 ± 9.3
M.G.	65	Male	16.9 ± 4.7	30.3 ± 1.3	52.8 ± 7.4	29.6 ± 6.5
T.K.	59	Female	17.1 ± 3.9	29.9 ± 4.8	53 ± 2.2	28.1 ± 5.4
M.F.	71	Male	16.6 ± 0.8	35.8 ± 9.0	47.6 ± 1.6	31.3 ± 7.2
E.B.	70	Male	16.1 ± 1.2	37.1 ± 8.3	46.8 ± 1.0	30.6 ± 6.9
J.C.	45	Female	18.2 ± 2.5	26.4 ± 5.5	55.4 ± 4.5	24.8 ± 7.0
Mean ± SD	57		17.6 ± 2.8	29.9 ± 4.9	52.5 ± 4.2	28.2 ± 6.8

In the second-stage operation, which was performed 8 months after the grafting procedure, a new flap was raised to expose the alveolar ridge, and 49 Straumann implants were placed (Figures 3 and 4).

Horizontal bony cylinders were harvested from the lateral window using a 3-mm diameter trephine under abundant saline irrigation for histological examination (Figures 5 and 6). To be sure that the biopsy region was the same in each patient, we harvested the bony cylinders in the first molar area at 8 mm from the crestal level directly into the newly formed bone without any contact with the old residual bone. At the end of the surgery, the mucosa was sutured with 4-0 silk. Amoxicillin (1 g twice daily for 6 days) was prescribed, and analgesics were given as needed. Three months elapsed before the implants were prosthetically loaded (Figure 8).

Tissue preparation

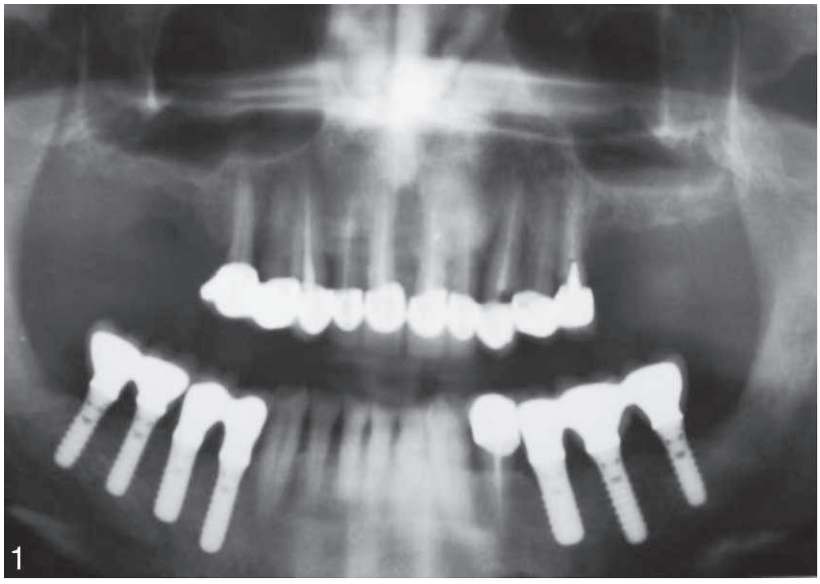
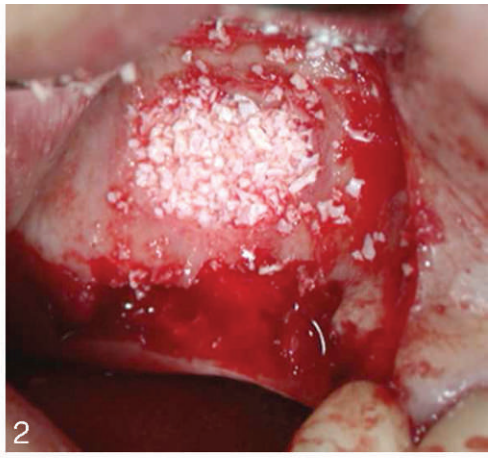
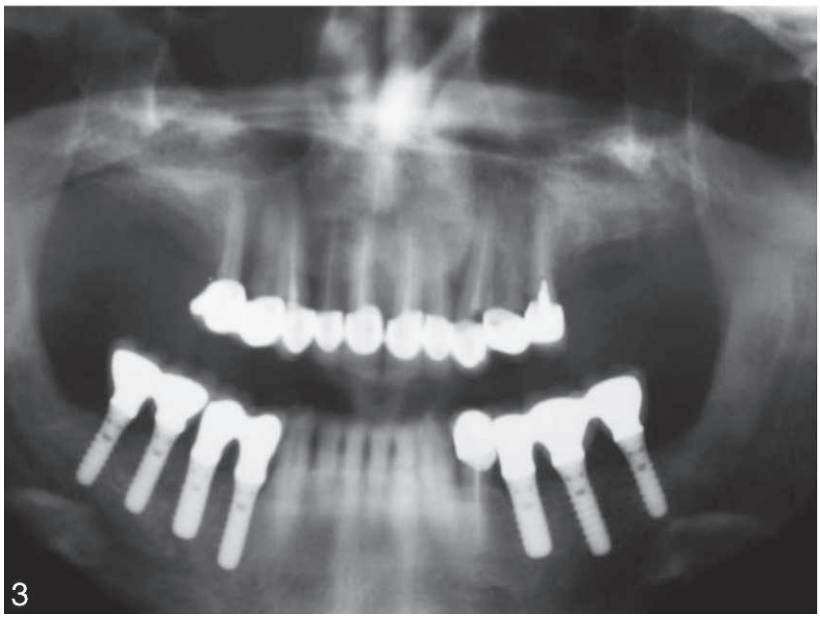
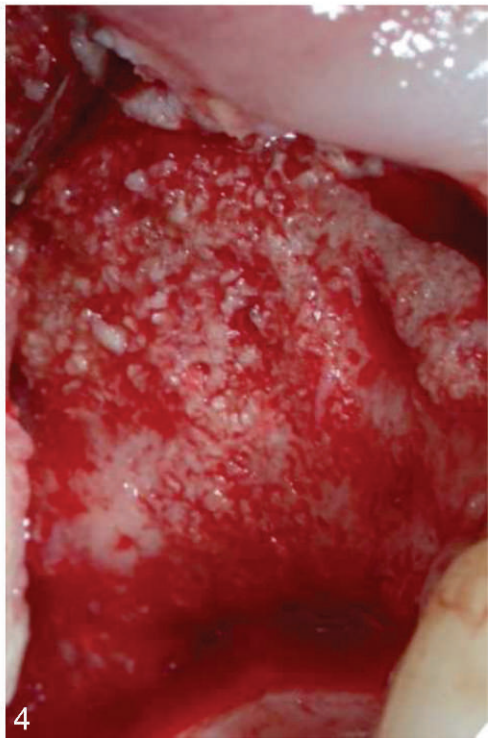
After dehydration, the biopsies were embedded without demineralization in methyl methacrylate (Merck, Darmstadt, Germany) and polymerized at −20°C for 48 hours. They were then processed for sectioning in a Polycut E microtome (Leica, Wetzlar, Germany). Serial sections 4-μm thick were cut for image analysis.

A series of 10 consecutive sections were subjected to histomorphometrical analysis. The sections were stained with toluidine blue (pH 3.8) and examined at a constant magnification (×260) with a semiautomatic image analyzer (Image tool 3.00 Uthscsa, San Antonio, Tex) coupling the microscope (Laborlux S; Leitz, Wetzlar, Germany) to a video camera (Olympus E330, Tokyo, Japan) and a computer (Figure 7). Data and terminology were standardized according to the recommendations of the American Society for Bone and Mineral Research nomenclature committee.[18]

The histomorphometrical values measured were (1) total bone volume, the percentage of the grafted area, consisting of bone tissue; (2) remaining Bio-Oss volume, the percentage of the grafted area consisting of deproteinized bovine bone (DPBB) material; (3) soft tissue volume, the percentage of the grafted area consisting of soft tissue; and (4) bone-DPBB contact surface, DPBB granules surface covered with bone, expressed as a percentage of the total DPBB granules surface.

Results

None of the 20 patients experienced postoperative sinus complications (eg, sinus congestion, graft infection, poor wound healing), and all implants were clinically integrated. Eight months after

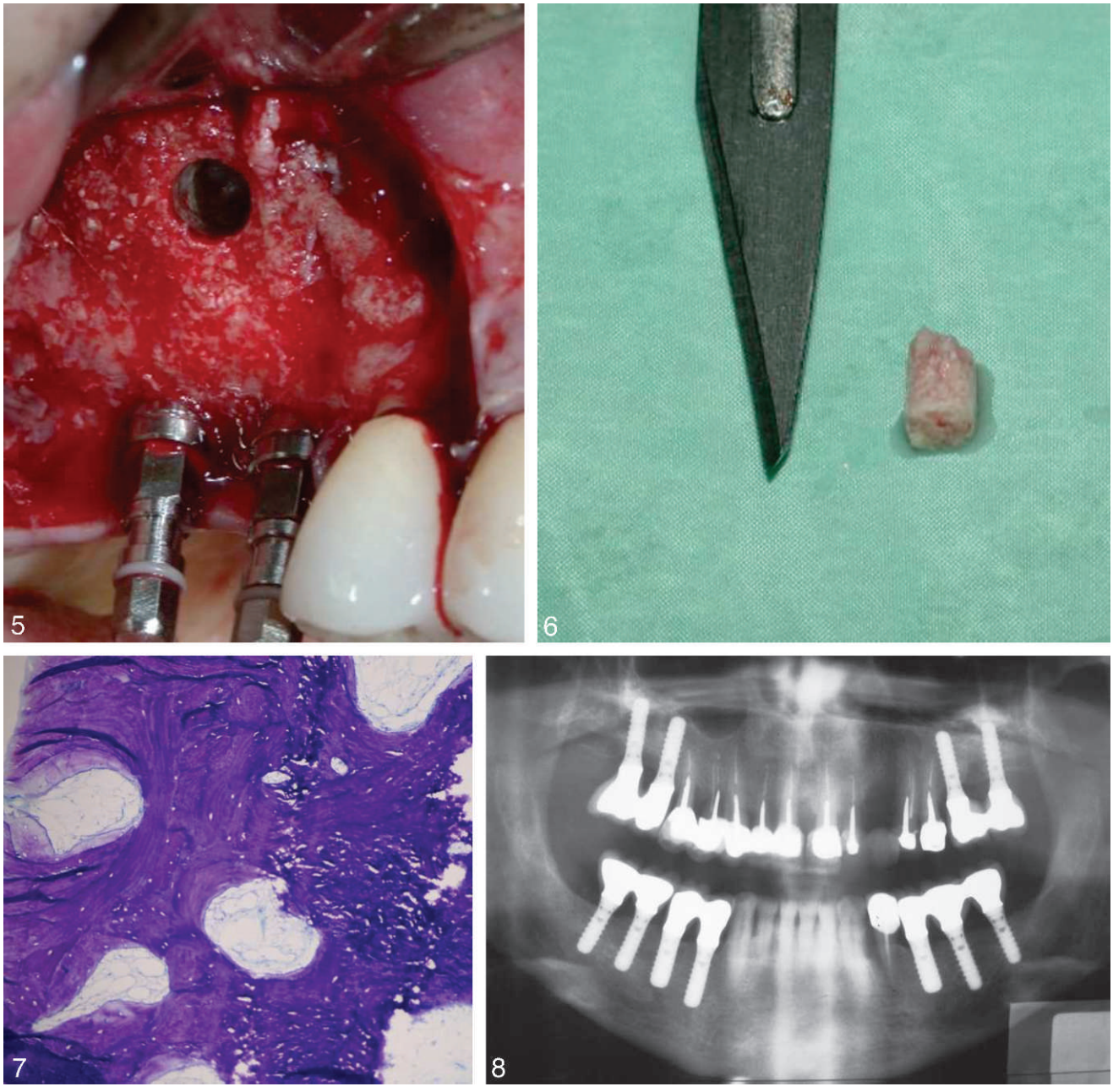





Figures 1–4. Figure 1. Preoperative panoramic radiography. The vertical bone supply in the posterior region of the maxilla is insufficient for implant placement. **Figure 2.** After elevating the Schneiderian membrane without perforation, xenograft is applied into the maxillary sinus. **Figure 3.** Panoramic radiograph 8 months after augmentation of the maxillary sinus floor. **Figure 4.** Clinical outcome ahead of removal of biopsy and ahead of implantation.

placement, Bio-Oss particles were easily distinguishable from the other components of the graft. Most of the particles were surrounded by newly formed bone. This bone was mature and compact, and it was possible to observe the presence of osteons with Haversian canals and capillaries.

Numerous osteoblasts were present in some areas, indicating active new bone formation on the external surface of the particles. In most of the microscopic fields, the particles were united by newly formed bone. No gaps were present at the bone particle interface, and the bone was always in close contact with the particles. No inflammatory cells were present around the particles or at the interface.

This mature and compact bone contributed to the osseointegration of the biomaterial particles. The histomorphometrical analysis showed an average percentage of newly formed bone of 17.6% (±2.8%) and a proportion of residual bone substitute material of 29.9% (±4.9%) of the total biopsy area. Intimate contact between newly formed bone and Bio-Oss was detected along 28.2% (±6.8%) of



Figures 5–8. Figures 5 and 6. Test biopsies are harvested from the augmented region during the implant placement. **Figure 7.** Toluidine blue coloration. **Figure 8.** Panoramic radiography after sinus floor elevation, implant placement, and implant loading.

the particle surfaces. Also, the results show that in all cases the DPBB granules had been interconnected by bridges of vital newly formed bone.

Discussion

Maxillary sinus lift procedures are designed to promote the formation of sufficient vertical bone in the maxillary sinus before placement of dental implants.[5] The success or failure of treatment with osseointegrated implants in edentulous patients depends on the volume and quality of the newly formed bone in the augmented space.[19,20]

The structure of Bio-Oss consists of a wide interconnecting pore system that enables this material to serve as a physical scaffold for osteogenic cells, thus promoting the migration and subsequent attachment of these cells. Bio-Oss

contains pores of different sizes: macropores (300-1500 mm), micropores (the size of Haversian and vascular marrow canals), and intracrystalline spaces (3–26 nm),[21] resulting in an overall porosity of 70%–75% and a wide internal surface area of almost 100 m^2/g.[22]

Clinically, the high osteoconductive property of Bio-Oss has been widely demonstrated.[23–25] Numerous investigators have recognized that Bio-Oss is an appropriate synthetic material to use in the treatment of osseous defects and maxillary sinus augmentation.[26] The present study confirms the preexisting data because all patients treated with Bio-Oss implants after maxillary sinus augmentation procedures had a great clinical response. The use of Bio-Oss alone showed a 100% implant success rate after 12 months of loading. Radiographic evaluations of the osseointegrated implants taken at 12 months showed that all the implants were well integrated in the augmented maxillary sinus.

This finding is in agreement with Froum and colleagues,[27] who found similar implant success rates when bovine bone was used with or without autogenous bone. Moy and colleagues[7] reported that a combination of bovine bone and autogenous bone yielded better outcomes than other bone graft regimens. Velich and colleagues[28] compared autogenous bone, heterografts, exogenous bone, and synthetic materials used alone or in combination with growth factors or morphogenetic proteins for sinus lifting. They found no differences in outcomes among these materials. However, even if the survival rates achieved in this clinical study appear to be good, longer observation times are necessary to evaluate the long-term outcomes of the procedures described in this study.

Despite its clinical success, Bio-Oss has often been corroborated by histological and histomorphometrical findings.[29–31] In the present specimens, Bio-Oss particles were incorporated and interconnected by a scaffold of newly formed bone; therefore, the bone graft appeared to have acted as a scaffold along which new bone formed. Similar results have been obtained in various other experimental and clinical studies.[32–34]

In another study, Klinge and colleagues[35] showed that Bio-Oss, in particular, seemed to promote more early bone formation than other substitutes. The Bio-Oss particles appeared to have acted as an osteoconductive material in the augmented sinus, because newly formed lamellar bone was in close contact with the graft particles.[36] In all specimens, it was possible to observe close contact between Bio-Oss particles and newly formed bone, with no gaps at the interface. Similar histological results have been reported by other authors.[37,38] The positive osteoconductive properties of Bio-Oss may be documented by close contact between the material and newly formed bone.[39]

With regard to the histomorphometrical analysis, the overall amount of new bone formation in these specimens was about 17.6% (±2.8%). Also, the amount of bone substitute particles found in the biopsies was about 29.9% (±4.9%). These results correspond well to findings of other investigators who used natural bone mineral alone as a graft material in sinus floor elevation.[40] These data suggest that predictable bone formation can be achieved with the use of natural bone mineral.

Moreover, because Bio-Oss is deproteinized, biological risks are avoided.[9] This bovine bone was also found to be more effective than hydroxyapatite as a bone substitute,[34] and it appeared to support a more physiological remodeling toward native bone.[41] In addition, this inorganic bovine bone has demonstrated good biocompatibility that elicits no foreign-body reactions.[34,36]

Conclusion

The results of our study demonstrate that DPBB mineral Bio-Oss can be used in a safe and predictable way to perform sinus augmentations, and it confirmed the osteoconductive properties of this bone substitute. Our results concur with those from other studies.[6]

No negative effects have been found with the use of Bio-Oss for sinus augmentation in association with dental implants. The use of this material in advanced osseointegration procedures can bring benefits to bone regeneration without risk of infection or disease transmission. The observed histological integration of bovine apatite, together with the 100% survival rate at the time of implant loading, supports clinical application for patients. Long-term results under prosthetic loading will provide information about whether maxillary sinus augmentation using Bio-Oss can ensure a suitable implant site over the long term. Also, further studies should be carried out to immunocytochemically

characterize the bone–Bio-Oss interface to elucidate whether it contains determinants of the mineralization front and what major proteins are involved in this process.

Abbreviation

DPBB: deproteinized bovine bone

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