20-Year Follow-up in Maxillary Sinus Floor Elevation Using Bovine-Derived Bone Mineral: A Case Report with Histologic and Histomorphometric Evaluation

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Bovine-derived bone mineral demonstrated good osteoconductive properties as grating material for maxillary sinus floor elevation, but the long-term behavior of this material has not been reported. The purpose of this report was to analyze and compare histomorphometric measurements of new bone, bone graft, and medullar spaces 6 months, 12 months, and 20 years after grafting. In the grafted area, the amount of mineralized bone was 16.96% at 6 months, 22.53% at 12 months, and 22.05% at 20 years, respectively. The amount of bovine-derived bone mineral ranged from 35.87% to 4.85% in the same period. The volume of the newly formed mineralized bone does not increase over time, conversely to nonmineralized bone. Int J Oral Maxillofac Implants 2018;33:1345–1350. doi: 10.11607/jomi.6884

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The use of bovine-derived bone mineral (BDBM) (Bio-Oss, Geistlich) as graft material for the maxillary sinus is now widely documented in the literature.1–4 When the lateral approach to the maxillary sinus is used, the phenomenon of osteoconduction leads to formation of vital bone, allowing long-term osseointegration of endosseous implants.3,5 Although many publications report the effectiveness of this material for producing persistent vital bone,6,7 the literature lacks information on the very long-term behavior of this material in this indication. The goal of the present case report is to describe the behavior of inorganic bovine bone over a period of 20 years.

CASE REPORT

This was approved by the Ethics Committee of the University of Corsica.

Surgical Procedure

In 1995, a patient aged 67 years was treated for the edentulous area of the right posterior maxilla following periodontitis, which caused loss of the premolars and molars. This patient was included in a 5-year study1 involving 15 patients with posterior maxillary tooth loss, for which a maxillary sinus graft via the lateral approach was indicated in order to be able to place implants during a second session.

For this patient, the bone height of the sinus floor was 2.2 mm. The graft was performed on September 28, 1995, with the technique described below. A preoperative computed tomography (CT) scan verified absence of any pathology of the maxillary sinus, the permeability of the ostium, and the absence of septa.

The patient was pretreated with a combination of amoxicillin and clavulanic acid, whose administration started 1 hour before surgery and continued for 1 week at a dosage of 1.5 g per day. At the end of the procedure, the patient received an intramuscular injection of 120 mg of prednisolone. Mouthwashes with 0.12% chlorhexidine were prescribed, as well as a combination of paracetamol and codeine to control postoperative pain.

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Periapical and palatal local anesthesia with a vasoconstrictor was performed.

A crestal incision shifted palatally was performed along the edentulous area up to the palatal surface of the canine. It was continued by an intra-sulcular incision to the level of the mesial surface of the canine, where a vertical discharge incision was used to elevate a full-thickness flap in order to expose the vestibular wall of the sinus cavity.

An elliptically shaped window was subsequently created using a diamond round bur mounted on an air turbine. The presence of an antral alveolar artery with intraosseous course resulted in significant bleeding, which was controlled by compression with a swab soaked in tranexamic acid.

The vestibular bone flap was subsequently mobilized and pushed back inside the sinus cavity thanks to the detachment of the sinus membrane with hand curettes. This detachment had been preceded by a perforation of the membrane; it was repaired with a collagen membrane (Bio-Gide, Geistlich).

When the anterior and medial walls of the sinus cavity were visible and only the flap was in a horizontal position, the graft could be inserted. BDBM mixed with physiologic saline was loaded into a 1-mL insulin syringe, the tip of which had been cut. The posterior part of the sinus was grafted first, followed by the anterior part and finally the central part. The material was systematically deposited in contact with the bone exposed by dissection of the membrane. It was slightly compressed to be completely soaked with blood. The mucoperiosteal flap was subsequently folded back and closed primarily, using several horizontal mattress sutures.

Two days after the surgery, the patient reported new bleeding and loss of granules through the mesial discharge incision. It was certainly a resumption of the bleeding of the antral alveolar artery after being injured while the vestibular osteotomy was being performed.

On February 29, 1996, after a healing period of 6 months (Fig 1a), three IMZ Frihex implants were placed in positions of the right maxillary premolars and first maxillary molar. During the preparation of the implant site at the position of the first maxillary molar, a biopsy specimen was taken using a trephine with a diameter smaller than that of the implant, and stored in 4% formaldehyde.

On September 20, 1996, at the end of an osseointegration period of 6 months, the implants were exposed and healing abutments placed. A second biopsy specimen in the position of the right second molar tooth (Fig 1a), always perpendicular to the ridge, was then taken and treated as before. A provisional fixed dental prosthesis was placed in sub-occlusion for 3 months in order to carry out a progressive loading. A definitive screw-retained metal ceramic fixed dental prosthesis was inserted.

Comparative histologic and histomorphometric analysis between the two biopsy specimens was performed. The patient was then followed regularly for 13 years and presented again only on April 7, 2015, because he complained of pain at the level of his fixed dental prosthesis replacing the first and second maxillary premolar.

The objective clinical examination revealed a 7-mm pocket depth in vestibular of the implant in the second premolar area and suppuration. This was described as a peri-implantitis requiring surgical treatment, which was performed on April 20, 2015. With the patient’s consent, at the time of surgery, 20 years after the graft (Fig 1b), two new biopsy specimens were realized. The first was taken in the position of the right maxillary third molar perpendicular to the ridge, and the second in the position of the first maxillary molar but perpendicular to the vestibular wall, where the window was located at the time of the graft in 1995.

The two biopsy specimens were preserved in the same manner and sent to the laboratory for histologic and histomorphometric analyses. It was thus possible to compare these biopsy specimens with those made in 1996 (Fig 2).

HISTOLOGY AND HISTOMORPHOMETRIC ANALYSIS

Histologic Processing

After retrieval of the tissue samples, they were fixed by immersion in 4% formalin. After decalcification
in 4.13% disodium ethylenediaminetetraacetic acid (EDTA) at 4°C for 6 weeks, the demineralized samples were washed in 0.1 M sodium cacodylate buffer containing 5% sucrose, pH 7.3, and subdivided into smaller samples and processed for embedding in LR White resin (Fluka) and paraffin.

Five-micron-thick paraffin sections were cut with a Reichert-Jung 2050 microtome and stained with hematoxylin and eosin. Semi-thin resin sections (1-µm-thick) were cut with a diamond knife on a Reichert Ultracut E microtome and stained with toluidine blue and basic fuchsin. Digital photography was performed using a ProgRes C5 digital camera (Jenoptik Laser, Optik Systeme) connected to a Zeiss Axioplan microscope (Carl Zeiss) and a digital camera (Axiocam MRx, Carl Zeiss) connected to an Axio Imager M2 microscope (Carl Zeiss).

Histomorphometry

The histomorphometric measurements were carried out in the augmented region of trabecular bone by point counting. The number of hits was converted into the percentage of areas occupied by bone, BDBM, or bone marrow space, thus representing the area fractions of these three compartments.

RESULTS

Biopsy Specimen No. 1

The biopsy specimen from the bone crest region consisted of three different compartments (Fig 2a): a small area (1) corresponding to the native bone below the sinus cavity, a large area (2) corresponding to the grafted area, and on top of it (3) some soft tissue corresponding to a sinus membrane remnant. The large area occupied by trabecular bone with fatty bone marrow consisted of bone and BM particles that were partly incorporated in the spongy bone and interconnected by bone (Figs 2b and 2c).

Active sites of bone formation were not present, and the surface of the BDBM particles was rounded. A few multinucleated giant cells were present at the interface of BDBM and bone marrow (Fig 3). However, signs of active resorption were not present, and a darkly stained seam was omnipresent at the surface of BDBM.
The area fractions of mineralized bone, bone marrow, and BDBM were 22.05%, 73.10%, and 4.85%, respectively (Table 1). For the nongrafted zone, the area fractions of bone and bone marrow were 11.75% and 88.25%, respectively (Table 2).

**Biopsy Specimen No. 2**

The biopsy specimen harvested from the buccal bone wall consisted of two different parts, i.e., a thin cortical bone layer and trabecular bone of low bone density containing mature fatty bone marrow (Fig 4). The grafted area consisted of bone, bone marrow, and BDBM particles that are partly incorporated in the spongy bone and interconnected by bone. Incorporated BDBM particles were present up to the external surface of compact bone. The surface of the BDBM particles was rounded. Active sites of bone formation were not present. A few multinucleated giant cells were present on the surface of BDBM exposed to the bone marrow space (Fig 5). However, signs of active resorption were not present, and a darkly stained seam was omnipresent at the surface of BDBM. The area fractions of mineralized bone, bone marrow, and BDBM were 30.68%, 57.15%, and 12.17%, respectively (Table 1).

**DISCUSSION**

It is interesting to compare the results of this biopsy specimen with those of the 1996 biopsy specimens. Comparison at the macroscopic level (Fig 6) shows that the difference in density between the grafted zone and the nongrafted zone decreases with time. After 20 years, it is difficult to differentiate between these two zones.
areas, apart from the presence of bovine bone residues in the grafted area.

In this region, at the quantitative level (Table 3), there is a reduction in the relative amount of bovine bone over time, matching the fact that the presence of multinucleated cells already identified as being osteoclasts is found.1

With regard to the mineralized part of the newly formed bone, the occupied volume increased appreciably between 0 and 6 months and then stabilized. Compared with that of the nongrafted zone, it was still greater at the time of biopsy sampling, whereas that of the nongrafted zone was reduced at 20 years. This can be attributed to bone ageing, which is always accompanied by demineralization and increased presence of fatty tissue.8,9 This difference may be because the newly formed bone is younger than the native bone.

At the same time, an increase of the volume occupied by medullary spaces between 1 year and 20 years was seen (Tables 2 and 3). Hence, like in the study by Sartori et al,6 it may be concluded that, although the amount of bone increases over time, it is only the amount of nonmineralized bone tissue (bone marrow) that increases, while the amount of mineralized tissue remains stable and tends to decrease over time because of an ageing phenomenon.8,9 The biopsy specimen perpendicular to the vestibular wall shows that the cortex is completely restructured. It may be assumed that there is a restoration “ad integrum” of the bone tissue, as it had existed before the tooth loss. There appears to be a morphologic similarity between newly formed and native bone; the general appearance is identical in terms of density, although histomorphometry shows it to be higher in the grafted area. Indeed, it appears that in an area where the bone is naturally type 3 or 4, it is impossible to obtain type 2 bone.

ACKNOWLEDGMENTS

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Table 3  Histomorphometry for the Grafted Area at Three Different Time Points

<table>
<thead>
<tr>
<th>Time</th>
<th>NB (%)</th>
<th>BM (%)</th>
<th>BDBM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>16.97</td>
<td>47.16</td>
<td>35.87</td>
</tr>
<tr>
<td>12 mo</td>
<td>22.53</td>
<td>46.96</td>
<td>30.51</td>
</tr>
<tr>
<td>240 mo</td>
<td>22.05</td>
<td>73.01</td>
<td>4.85</td>
</tr>
</tbody>
</table>

NB = new mineralized bone; BM = bone marrow; BDBM = bovine-derived bone mineral.
REFERENCES


