The Efficacy of Demineralized Bone Matrix and Cancellous Bone Chips for Maxillary Sinus Augmentation

Demineralized bone matrix and cancellous bone chips in a reverse-phase medium carrier (DynaBlast, Keystone Dental) were used to augment the maxillary sinuses in 8 patients requiring 10 sinus augmentations. Clinical reentry after 6 to 7 months (mean, 6.2 months) and computed tomographic scan evaluation at 5 months demonstrated new bone formation as well as sufficient radiopaque volume to place implants in all sites. Microcomputed tomographic evaluation and histomorphometric analysis of sinus core biopsies confirmed the formation of new bone and demonstrated three distinctive mineralization patterns that have been previously described. DynaBlast can be considered a viable alternative to the use of autogenous bone or other types of grafting materials. (Int J Periodontics Restorative Dent 2009;29:415–423.)

1Assistant Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.
2Assistant Clinical Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.
3Private Practice, Institute for Advanced Dental Studies, Belo Horizonte, Brazil.
4Adjunct Professor, Department of Periodontics, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
5Private Practice, Swampscott, Massachusetts.
6Lecturer, Department of Periodontology, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
7Associate Clinical Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.

Correspondence to: David M. Kim, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, MA 02115; fax: +617-432-1897; email: dkim@hsdm.harvard.edu.

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DFDBA is frequently combined with other grafting materials to improve bone density.\textsuperscript{10–18}

This study evaluated the use of DynaBlast (Keystone Dental) to augment the maxillary sinus for the purpose of placing dental implants to restore posterior occlusion. The product consists of DBM and cancellous bone chips in a reverse-phase medium (poloxamer 407). This combination is thought to be advantageous in that DBM acts to stimulate new bone growth, and the cancellous bone chips offer an osteoconductive scaffold for bone deposition and remodeling. Poloxamer 407 is an inert ingredient that has been used in over-the-counter pharmaceuticals, and the exceptional handling property of DynaBlast depends on this unique carrier. This temperature-responsive reverse-phase medium acts to enhance both handling and graft containment. The present study employed histomorphometric analysis together with light microscopy, computed tomography (CT), and microCT analysis to demonstrate both new bone formation and the degradability of the matrix.

Method and materials

The study was conducted at two sites in a total of eight patients (ages 31 to 62 years). None of the patients had significant systemic disorders and all required a buccal lateral window sinus augmentation procedure. An informed consent document was reviewed with each patient and signed prior to treatment. Preoperative assessment by periapical radiographs and CT scans revealed insufficient native alveolar bone height (less than 6 mm) for implant placement without sinus augmentation. The procedure was carried out bilaterally in two patients and unilaterally in six patients.

The surgical procedure was performed under local anesthesia (2% lidocaine with 1:100,000 epinephrine). First, a mucoperiosteal flap was elevated to expose the buccal bone of the maxillary sinus. An oval osteotomy was created with a piezoelectric surgical device (Piezosurgery, Mectron). The integrity of the sinus membrane was preserved, and the membrane was elevated to the medial wall of the sinus (Fig 1). The cavity was then filled with DynaBlast in several steps, and the area was covered with an absorbable collagen membrane (Bio-Gide, Osteohealth) (Figs 2 and 3). Primary flap closure was accomplished with 3-0 silk sutures (Ethicon Inc). The patients received antibiotics (amoxicillin, 500 mg 3 times daily for 5 days, or clindamycin, 300 mg 3 times daily for 5 days) and analgesics (ibuprofen, 800 mg 3 times daily for 5 days). They were also instructed to rinse twice daily with 0.12% chlorhexidine digluconate solution for 1 week. They all received written postoperative instructions and were advised to return in 7 to 10 days.
for suture removal and wound assessment. They were monitored monthly for the next 6 to 7 months.

A postoperative CT scan was obtained 5 months after sinus augmentation to determine the gain in bone height; this was done by subtracting the height of the native bone from the total new bone available. Sinus core biopsies (both lateral and crestal) were obtained using a 3-mm trephine bur (Biomet 3i) at 6 to 7 months. They were immediately placed in formalin and shipped to a histologist for microCT, histologic, and histomorphometric evaluations.

Sixteen dental implants (Certain Prevail, Biomet 3i) were placed into the osteotomy site created from biopsy sampling.

**MicroCT**

The fixed cores were scanned using a high-resolution microCT system (µCT 40, Scanco Medical) in a multislice mode. Each image data set consisted of approximately 600 microCT slice images. The specimens were scanned in high-resolution mode with an x-, y-, z-resolution of 12 µm. The image data sets were used to produce three-dimensional (3D) views of the specimens with special software (Scanco Medical).

**Light microscopy**

Histologic processing was performed on eight biopsies. The cores were embedded following complete dehydration in ascending grades of ethanol in a light-curing one-component composite resin (Technovit 7200 VLC, Heraeus Kulzer). Polymerized blocks were initially ground to bring the tissue components closer to the cutting surface. A 100-µm-thick section attached to the second slide was sawed with a diamond blade and 50 to 100 g of pressure. The final thickness of 40 µm was achieved by grinding and final polishing with 1,200-, 2,400-, and 4,000-grit sandpaper. Sections from each block were used for toluidine blue/pyronine G staining without deplastination.

**Histomorphometry**

With specialized software (Scanco Medical), a threshold was set to distinguish the grey levels of newly formed bone versus cancellous bone chips. Based on these values, the percentages of both new bone and cancellous bone chips were calculated.

The microCT evaluation discriminated between two main grey levels. The brighter areas, ie, indicating a slightly higher mineralization, were defined as representing cancellous bone chips, and the darker grey areas represented newly formed bone. Based on these grey levels, 3D reconstructions were created showing a longitudinal cut through the core. To ease viewing of the two selected grey levels, false colors were chosen (white for cancellous bone chips and red for bone).
Results

Clinical and CT evaluations

Ten sinus augmentations were performed in eight patients. The CT scan evaluation at 5 months after sinus augmentation demonstrated an increase in radiopaque volume in the sinus (Figs 4a and 4b). Clinical reentry at 6 to 7 months (average 6.2 months) showed new bone formed at the site of the surgical osteotomy that was indistinguishable from the neighboring native bone (Fig 5). The biopsy samples in some areas (five sites) revealed soft bone upon reentry. Sixteen dental implants were placed in augmented areas, and they are in the process of being restored at this time.

MicroCT and histomorphometric analyses

A threshold was set to distinguish newly formed bone from cancellous bone chips. Based on these values, the percentages of both new bone and cancellous bone chips were calculated. The microCT evaluation revealed the presence of densely mineralized cores composed of newly formed bone and cancellous bone chips (Figs 6a and 6b).
Bone formation was evident to varying degrees in all specimens. However, the microCT evaluation could not differentiate DBM from bone marrow or connective tissue.

Quantitative assessment of new bone, remaining graft particles, and marrow space/connective tissue was done on eight specimens. The average percentage of newly formed bone at 6.2 months was 23.8% ± 4.2% (Table 1). The average percentage of cancellous bone chips (CBCs) was 9.1% ± 8.1%. The average percentage of DBM/marrow spaces/connective tissue was 67.2% ± 9.4%.

**Light microscopy**

The ground sections of specimens revealed various amounts of newly formed bone tissue, residual DBM particles and CBCs, and loose connective tissue (Fig 7). In most specimens, a distinctive mineralized tissue distribution pattern was noted. A high percentage of mineralized tissue volume was identified near maxillary native bone, while residual DBM particles were present away from the maxillary native bone. Interestingly, residual CBCs were presented throughout the cores.

<table>
<thead>
<tr>
<th>Patient</th>
<th>New bone (%)</th>
<th>CBC (%)</th>
<th>DBM/marrow/CT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>27.7</td>
<td>5.7</td>
<td>66.6</td>
</tr>
<tr>
<td>Patient 2</td>
<td>24.1</td>
<td>5.6</td>
<td>70.3</td>
</tr>
<tr>
<td>Patient 3</td>
<td>23.8</td>
<td>27.4</td>
<td>49.9</td>
</tr>
<tr>
<td>Patient 4</td>
<td>17.7</td>
<td>3.1</td>
<td>79.2</td>
</tr>
<tr>
<td>Patient 5</td>
<td>21.8</td>
<td>6.1</td>
<td>72.1</td>
</tr>
<tr>
<td>Patient 6</td>
<td>26.1</td>
<td>14.4</td>
<td>59.5</td>
</tr>
<tr>
<td>Patient 7</td>
<td>29.9</td>
<td>4.7</td>
<td>65.4</td>
</tr>
<tr>
<td>Patient 8</td>
<td>18.9</td>
<td>6.2</td>
<td>74.9</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>23.8 ± 4.2</td>
<td>9.1 ± 8.1</td>
<td>67.2 ± 9.4</td>
</tr>
</tbody>
</table>

CBC = cancellous bone chips; DBM = demineralized bone matrix; CT = connective tissue.

Fig 7  Ground section of 7-month specimen showing maxillary native bone, cancellous bone chips (CBC), demineralized bone matrix (DBM), marrow space, and new bone.
Residual DBM particles and CBCs, along with newly formed bone bridging the space between them, were noted near the maxillary native bone. New bone in this area seemed to be directly apposed to the surfaces of graft particles, with both DBM particles and CBCs serving as scaffolds for this new bone growth.

Bone formation around CBCs and in particular by remineralization of DBM was evident on the cranial side of the cores. Detailed histologic evaluations of whole cores revealed three different patterns of mineralization, which have been previously described by Groeneveld et al. The first mineralization pattern was accompanied by the presence of an osteoid and osteoblasts in conjunction with new bone formation in the biopsy area near the native maxillary bone and the adjoining grafted area (Fig 8). Both woven and lamellar bone was observed. The second mineralization pattern was seen as a remineralization phenomenon within the DBM particles. In this case, remineralization seemed to start in the particle center, whereby the mineralization front was following the lamellar organization of the DBM particle (Fig 9). As the space between DBM particles was bridged by newly formed bone formed by the first mineralization pattern, a continuous pattern of mineralization appeared. Early stages of this pattern were observed in areas independent of the presence of living bone tissue. The third mineralization pattern was observed in areas where vital bone was formed next to DBM particles. Outgoing from contact points with vital bone, remineralization of DBM particles occurred in small isolated mineralized dots (Fig 10). Remineralization usually started at the periphery of DBM particles and advanced toward the inner area. In addition, DBM particles undergoing acellular remineralization showed a repopulation of the bone lacunae by new osteocytes. Backscatter scanning electron microscopic evaluation of particles undergoing remineralization confirmed the presence of mineralization (Fig 11).

Fig 8 (left) New bone (NB) can be seen along both DBM and CBCs, forming bridges between these two biomaterials.

Fig 9 (right) Remineralization of DBM follows the lamellae of the demineralized chips.

Fig 10 (left) DBM undergoing acellular remineralization. A repopulation of the bone lacunae by new osteocytes can also be seen.

Fig 11 (right) Backscatter scanning electron microscopy of a DBM particle (area shown in Fig 10) undergoing acellular mineralization.
Discussion

The sinus elevation procedure has proven to be an effective means to create bone of sufficient volume and quality to place osseointegrated implants and restore them to function. Recent evidence supports the use of autogenous bone substitutes such as allografts and xenografts, providing clinicians with a range of safe and effective biomaterials for regenerative therapies.3–6

The present study has confirmed that DynaBlast produced a sufficient volume of new bone when placed in the maxillary sinus. The material consists of DBM and CBCs in a reverse-phase medium carrier. A healing period of 6 to 7 months allowed incorporation of the grafts, with subsequent maturation of newly formed bone tissue and significant vascularization.

Clinical, CT, and microCT evaluations all provided evidence of new bone formation. Descriptive histologic analysis confirmed that new bone was forming in three distinctive patterns, as previously described by Groeneveld et al.19 New bone was formed along the border of both CBCs and DBM, bridging the spaces between them. In addition, an acellular remineralization of the DBM either followed the lamellar pattern of the DBM or occurred as a spotlike front of remineralization starting at the border of the DBM and advancing to the inner areas.

The percentage of new bone formed in the present study was equivalent to that described in similar studies.20–22 Histomorphometric analyses in humans have revealed that new bone formation in sinus areas varies considerably, depending on bone graft type and healing time.23 For example, Lee et al reported a higher percentage of vital bone formation in sinuses augmented with bovine hydroxyapatite at 12 months than at 6 months.23 Hanisch et al evaluated new bone formation at various times following sinus augmentation with a composite DFDBA–bovine hydroxyapatite combination graft (ratio of 1:1 by volume); they demonstrated that new bone formation at 12 months (20.7% ± 8.3 %) was significantly greater than at 6 months (8.1% ± 3.0%) and 8 months (9.0% ± 3.8 %).12 These findings suggest that the present authors could have obtained a higher percentage of new bone formation if more than 7 months had been allowed to elapse.

Traditionally, DFDBA has been combined with other biomaterials to support new bone formation in the sinus. Nevertheless, the addition of other bone substitutes reduces the amount of DBM that is present per unit volume, and this may have negative consequences for osteoinductivity.24 Combinations of biomaterials have resulted in various percentages of new vital bone, nonvital residual grafting materials, and connective tissue. The combination of putty containing human DFDBA in hyaluronic acid (DBX, MTF) with beta-tricalcium phosphate was found to be less effective than a combination of DFDBA with anorganic bovine bone graft in sinus elevation procedures in terms of the amount of new bone formed and implant insertion torque.18

It has been reported that the preservation of growth factors during
demineralization is challenged, and there is great variability in the osteoinductivity of different batches of commercially available DFDBA. Close study of DBM will help to determine its osteogenic potential when combined in paste, putty, or gel.

Conclusion

The use of DynaBlast to augment bone in the floor of maxillary sinuses created sufficient bone in all treated sites for implant placement and can be considered as a viable alternative to the use of autogenous bone or other types of grafting materials. The present results demonstrated that the reverse-phase medium carrier can be used with demineralized bone matrix and cancellous bone chips without reducing the clinical effectiveness of the allograft.

Acknowledgment

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References


