Histomorphometric Analysis of Newly Formed Bone After Maxillary Sinus Floor Augmentation Using Ground Cortical Bone Allograft and Internal Collagen Membrane

Roni Kolerman,* Haim Tal,* and Ofer Moses*

**Background:** Maxillary sinus floor augmentation is the treatment of choice when insufficient alveolar bone height prevents placement of standard dental implants in the posterior edentulous maxilla. The objective of this study was to histologically and histometrically evaluate new bone formation after maxillary sinus floor augmentation using ground cortical bone allograft.

**Methods:** Mineralized freeze-dried bone allograft (FDBA) was used for sinus floor augmentation. After 9 months, 23 biopsies were taken from 19 patients. Routine histologic processing using hematoxylin and eosin and Mallory staining was performed.

**Results:** Histologic evaluation revealed a mean of 29.1% newly formed bone, 51.9% connective tissue, and 19% residual graft material. Graft particles were mainly in close contact with newly formed bone, primarily with features of mature bone with numerous osteocytes, and, to a lesser extent, with marrow spaces. There was no evidence of acute inflammatory infiltrate.

**Conclusion:** FDBA is biocompatible and osteoconductive when used in maxillary sinus-augmentation procedures, and it may be used safely without interfering with the normal reparative bone process. *J Periodontol 2008;79:2104-2111.*

**KEY WORDS**
Allograft; collagen; membrane; sinus.

Insufficient alveolar bone height often prevents placement of standard dental implants in the posterior edentulous maxilla. This lack of bone height may be the result of alveolar bone loss, sinus pneumatization, or both.¹ Maxillary sinus augmentation compensates for this pathological condition by increasing alveolar bone height prior to or simultaneous with endosseous implant placement.²,³ Sinus floor elevation procedures facilitating implant placement in the severely atrophic posterior maxilla were first presented by Tatum in the late 1970s and published shortly thereafter by Boyne and James⁴ in 1980. The technique was modified repeatedly.⁵-10

A sinus lift procedure adequately increases the vertical dimension of the resorbed alveolar process in the posterior maxilla, thus enabling placement of implants of sufficient length at this site. Grafting materials, including autogenous bone,⁴,⁵ demineralized freeze-dried bone allograft (DFDBA),¹¹-¹⁴ mineralized freeze-dried bone allograft (FDBA),¹⁴ xenografts,¹⁵-¹⁸ hydroxyapatite preparations,⁵,¹⁵ calcium sulfate preparations,¹⁹,²⁰ as well as growth factors embedded in different carrier materials,²¹-²³ have been used successfully to augment the floor of the maxillary sinus.

* Department of Periodontology, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel.

It was reported that DFDBA has a limited effect on sinus augmentation bone growth. Only a limited number of human histomorphometric studies investigated the use of DFBA in sinus floor augmentation procedures.

The present study was conducted to clinically, histologically, and histomorphometrically evaluate the regenerative potential of DFDBA in sinus lift procedures.

**MATERIALS AND METHODS**

Participants were selected from a pool of patients who required sinus elevation procedures for the placement of posterior implants. The patients were treated in the clinics of Clalit Sick Fund, Tel-Aviv, Israel by one of the authors (RK) between 2003 and 2006. Patients were informed about alternative treatment plans and selected the plan requiring maxillary sinus elevation.

Patients who presented with a moderately or severely atrophic posterior maxilla with ≤6 mm of residual alveolar bone were selected for the study. Patient exclusion criteria were chronic steroid therapy, uncontrolled diabetes, cardiovascular disease, past irradiation of head and neck, maxillary sinus cysts, active chronic sinusitis, or smoking more than five cigarettes per day during the 3 months preceding the study. The study population included 19 adult patients (eight men and 11 women) ranging in age from 43 to 78 years (average age for men, 57.37 years; women, 59.18 years, Table 1). Four patients had both sinuses elevated; thus, 23 sinuses were treated. A staged approach was carried out in all 23 sites; sinus lift grafting procedures were followed by implant placement 9 months later. Mineralized freeze-dried bone allograft (DFBA), 1 250 to 710 μm, was the filler material of choice. Biopsies were harvested from the proposed implant sites immediately prior to implant placement.

Participants signed an informed consent form in which the procedure was explained in detail according to the patient’s treatment plan. Procedures were performed according to the technique described by Smiler and Holmes. Briefly, a mucoperiosteal buccal flap was elevated exposing the lateral bony wall of the sinus antrum at the edentulous region distal to the first premolar. A round diamond bur, 2 mm in diameter, was used to outline the lateral window, which was completely removed to expose the Schneiderian membrane. Using a large flat curet, the membrane was gently separated from the housing bone and pushed away to achieve a tension-free reflection exposing the sinus walls. Prior to graft placement, an inner occlusive native collagen membrane was placed underneath the reflected Schneiderian membrane and served as a “roof” for the augmented space. The membrane was adapted to make contact with the lateral bony walls, thus defining a space limited by peripheral bony walls, the osseous floor below, and an upper border created by a collagen barrier covering the Schneiderian membrane.

The established void was filled with 2.5 to 6 ml saline wet DFBA grafting material, followed by placement of the collagen membrane over the lateral window and primary soft tissue closure using 4/0 silk sutures. Postoperatively, systemic antibiotic (amoxicillin and potassium clavulanate, 875 mg, twice a day) was prescribed for 1 week, and naproxen sodium (one 275-mg tablet every 6 to 8 hours for 24 hours) was prescribed for pain control. Dexamethasone (4 mg daily) was administered for 2 days to minimize edema. Antiseptic mouthwash (0.2% chlorhexidine gluconate) was used twice daily (30 seconds each time) for 2 weeks. Sutures were removed after 14 days, followed by uneventful soft tissue healing.

At 9 months, postoperative CT scans were taken, demonstrating radiopaque areas inside the grafted maxillary sinus in all patients. Implant locations were determined according to the patient’s treatment plan. Immediately prior to implant placement, biopsies were harvested from the implant sites using trephine drills measuring 2.5 to 4 mm in diameter and 7 to 15 mm long. Biopsies included pristine and newly formed bone and were fixed in 10% neutral buffered formalin for 96 hours, decalcified in 5% formic acid for 14 days, and embedded in paraffin.

Blocks were cut to 5-μm-thick slides and stained with hematoxylin and eosin and Mallory stain. Each biopsied site was enlarged into a regular osteotomy, surgical antiseptic environment.

Surgical procedure

Patients were premedicated 1 hour before surgery with dexamethasone, 8 mg, and amoxicillin and potassium clavulanate, 875 mg. Local anesthesia included 3% lidocaine HCl (2 to 6 ml) and base norepinephrine (0.04 mg). Patients rinsed their mouths with 0.2% chlorhexidine gluconate solution for 1 minute immediately before surgery to obtain a better surgical antiseptic environment.

Surgical procedures were performed according to the technique described by Smiler and Holmes.
and implants (4, 5, or 6 mm in diameter, 12 to 16 mm long) were placed.

**Histomorphometric Analysis**
The analysis was carried out using a grid eyepiece with horizontal and vertical lines (creating 121 intersections) at ×200 magnifications. Each slide was measured at 10 sites, resulting in 1,210 readings per slide. Measurements were taken only from newly formed tissue; pristine bone was identified by the lack of graft material that simulates acellular bone and was excluded from the data analysis. Graft particles were identified by their typical appearance, especially the presence of empty lacunae. Analysis included bone

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Gender</th>
<th>New Bone</th>
<th>FDBA (filler)</th>
<th>Bone Marrow (ct)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>Female</td>
<td>25.68</td>
<td>11.21</td>
<td>63.11</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>Female</td>
<td>30.86</td>
<td>19.18</td>
<td>49.96</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>Female</td>
<td>25.62</td>
<td>33.55</td>
<td>40.83</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>Female</td>
<td>22.85</td>
<td>23.52</td>
<td>53.63</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>Female</td>
<td>29.34</td>
<td>9.67</td>
<td>60.99</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>Female</td>
<td>39.46</td>
<td>9.28</td>
<td>51.25</td>
</tr>
<tr>
<td>7</td>
<td>59</td>
<td>Female</td>
<td>20.28</td>
<td>9.72</td>
<td>69.99</td>
</tr>
<tr>
<td>8</td>
<td>62</td>
<td>Female</td>
<td>17.26</td>
<td>18.73</td>
<td>64.01</td>
</tr>
<tr>
<td>9</td>
<td>63</td>
<td>Female</td>
<td>31.88</td>
<td>18.08</td>
<td>50.04</td>
</tr>
<tr>
<td>10</td>
<td>72</td>
<td>Female</td>
<td>28.43</td>
<td>23.55</td>
<td>48.02</td>
</tr>
<tr>
<td>11</td>
<td>78</td>
<td>Female</td>
<td>50.25</td>
<td>8.82</td>
<td>40.92</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>Male</td>
<td>29.32</td>
<td>18.78</td>
<td>51.89</td>
</tr>
<tr>
<td>13</td>
<td>51</td>
<td>Male</td>
<td>40.83</td>
<td>9.42</td>
<td>49.75</td>
</tr>
<tr>
<td>14</td>
<td>52</td>
<td>Male</td>
<td>29.47</td>
<td>24.01</td>
<td>46.52</td>
</tr>
<tr>
<td>15</td>
<td>56</td>
<td>Male</td>
<td>31.74</td>
<td>22.78</td>
<td>45.48</td>
</tr>
<tr>
<td>16</td>
<td>57</td>
<td>Male</td>
<td>17.92</td>
<td>32.62</td>
<td>49.46</td>
</tr>
<tr>
<td>17</td>
<td>58</td>
<td>Male</td>
<td>23.40</td>
<td>23.81</td>
<td>52.79</td>
</tr>
<tr>
<td>18</td>
<td>66</td>
<td>Male</td>
<td>17.67</td>
<td>29.50</td>
<td>52.83</td>
</tr>
<tr>
<td>19</td>
<td>74</td>
<td>Male</td>
<td>43.00</td>
<td>9.00</td>
<td>48.80</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>Female</td>
<td>35.08</td>
<td>5.25</td>
<td>59.67</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>Male</td>
<td>33.87</td>
<td>25.05</td>
<td>41.08</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>Male</td>
<td>37.97</td>
<td>12.16</td>
<td>49.88</td>
</tr>
<tr>
<td>Average</td>
<td>58.74</td>
<td></td>
<td>29.09</td>
<td>19.00</td>
<td>51.91</td>
</tr>
<tr>
<td>Minimum</td>
<td>43</td>
<td></td>
<td>6.86</td>
<td>5.25</td>
<td>40.83</td>
</tr>
<tr>
<td>Maximum</td>
<td>78</td>
<td></td>
<td>50.25</td>
<td>39.26</td>
<td>69.99</td>
</tr>
<tr>
<td>SD</td>
<td>9.02</td>
<td></td>
<td>9.78</td>
<td>9.34</td>
<td>7.49</td>
</tr>
</tbody>
</table>

*ct = connective tissue.*
graft particles, newly formed bone, and connective tissue. Calculation of the percentage of each of the three components in the slide was achieved by counting the presence of each tissue type on every grid intersection. The total count of all points of each tissue type divided by 1,210 represented the relative percentage of each component involved in the area of the newly regenerated tissue.

**Statistical Analysis**
The Student *t* test and Pearson correlation test were used for statistical analysis of the data calculated based on the overall percentage of each component in each slide. Independent variables were age and gender; the dependent variables were soft tissue, residual graft, and new bone.

**RESULTS**
No complaints or reports of side effects were recorded postoperatively. Nine-month CT scans demonstrated additional vertical and horizontal radiopaque material measuring 6 to 18 mm in height above the sinus floor.

**Histology**
Newly formed bone and allograft particles were observed in all augmented sites. Allograft particles were identified by their typical structure by the presence of empty lacunae and separation lines. Allograft particles were in intimate contact with newly formed bone or connective tissue (Figs. 1 and 2). Osteoblasts were present in conjunction with newly formed bone around the FDBA particles. There was no evidence of acute or chronic inflammatory infiltrate.

**Histomorphometry**
Histomorphometric analysis of the 23 cores revealed that the percentage of new bone ranged from 6.86% (patient 15) to 50.25% (patient 9), with an average of 29.1% ± 9.78%. The average percentage of marrow and connective tissue was 51.9% ± 7.49%; residual graft particles averaged 19% ± 9.34% (Table 1). No histomorphometric differences were found between the genders (*P* = 0.545; Table 2), and no correlation was found with age (*P* = 0.359; Table 3).

**DISCUSSION**
The goal of this study was to evaluate healing 9 months following sinus grafting with ground cortical bone allograft covered by an internal collagen membrane underneath the elevated Schneiderian membrane. The
rationale for this evaluation was the assumption that different filling materials and surgical techniques may modulate the quality and quantity of newly formed bone in sinus lift procedures. Furthermore, it is expected that the use of an internal sub-Schneiderian collagen membrane, in addition to the commonly used "window" occluding barrier membrane, may have an additional favorable clinical effect in the event of unnoticed perforations of the Schneiderian membrane.

The percentage of new bone formation in the present study ranged between 6.86% and 50.25%, with a mean of 29.1%. The low percentage of new bone formation in patient 15 (6.86%) might have occurred as the result of a very large tear in the Schneiderian membrane. Although an internal collagen membrane was used to cover this tear, the tear might have occurred because of early resorption of the collagen membrane that was exposed to the sinus fluids or membrane shift away from the perforation during graft placement, with subsequent partial loss of graft material. Placing an extension of the internal collagen membrane outside the sinus window and stabilizing it with titanium tacks may avoid such accidents. A reduced percentage of new bone formation was shown in cases with accidentally perforated Schneiderian membranes compared to the contralateral side with intact membranes (14.17% versus 33.58%, respectively). Excluding patient 15 from the study increased the mean percentage of new bone formation to 30.09%.

The low percentage of new bone in patients 7, 13, and 14 (~17%) may be explained, in part, by the presence of very large sinus cavities mesio-distally and bucco-palatally; large sinuses may have a reduced osteogenic potential as a result of the large distance between the peripheral source of osteogenic cells and the examined biopsy sites. It was reported that angiogenesis plays a crucial role in guided bone regeneration. The vascular supply preceding osteogenesis during wound healing originates from the peripheral bony walls. Therefore, bone formation would be expected to expand from the peripheral bony walls toward the center of the graft.

New bone growth (29.1%) was lower in the present study compared to the values recorded by Cammack et al., who used FDBA (41%) or DFDBA (36%) as augmentation materials. The difference between the two studies may derive from the different morphometric techniques used. The present study used the point-counting technique, which uses an eye grid, whereas Cammack et al. interpreted results from a set of polygons traced on the sample, which allowed the software to calculate a set of areas, i.e., new bone residual graft particles and soft tissue as part of the total area of the sample. Another explanation may be the time of biopsy harvesting, which was 11 months in the previous study compared to 9 months in the present one. Thus, from a biologic standpoint, it may be assumed that the larger the sinus, the longer the maturation time required to achieve an acceptable percentage of new bone formation.

The present study is in agreement with Froum et al., who reported 28.3% vital bone using a mineralized cancellous bone allograft for sinus augmentation. These
studies support the claim that FDBA and DFDBA are osteoconductive materials. These data disagree with those found by Valentini and Abensur, 17 who showed that newly formed bone was in contact with deproteinized bovine bone mineral (DBBM) particles, whereas DFDBA particles were only surrounded by connective tissue.

The inability of DFDBA to induce new bone formation in sinus elevation procedures was one conclusion of the Sinus Consensus Conference of 1996.11 The efficacy of DBBM as a graft material for sinus floor elevation is well documented.15-17 In one study17 in which DBBM was used, newly formed bone increased from 21.1% to 27.6% between 6 and 12 months, whereas the graft particles decreased from 39.2% to 27%. The increased quantity of newly formed bone with time occurred mainly in the connective tissue component, with minimal change in the residual graft.17 A lack of breakdown of DBBM particles was observed by some investigations30-32 whereas other investigators33,34 reported signs of resorption and osteoclastic activity around DBBM particles.

In a split-mouth study15 that compared DBBM to a non-ceramic bioabsorbable hydroxyapatite graft, particles in all specimens were surrounded by newly formed bone in direct contact with the particles or by connective tissue from bone marrow. The new bone fraction was higher in the DBBM-grafted sinus (42.1%) compared to the non-ceramic bioabsorbable hydroxyapatite graft (33.3%), whereas the graft particle fraction was similar (24.7% versus 24.6%, respectively).15

In 20 human sinuses in which DBBM was used as a filler, Piattelli et al.35 found 30% bone, 30% residual graft particles, and 40% bone marrow 6 months after the procedure. These results are comparable to the present new bone formation values (29.1%). A systematic review36 of implant survival in maxillary sinus grafts that included 6,913 implants placed in 2,046 patients revealed an overall survival rate of 91.49%; 87.7% when autogenous bone alone was used, 94.88% when autogenous bone was combined with other materials, and 95.98% when bone substitutes were used alone. An analysis of the survival rate for each type of graft material was beyond the aim of that study.36 In contrast, a recent systematic review37 of the main database of implant survival with different graft materials used for sinus augmentation reported that the survival rate was 92% for implants placed in a mixture of autogenous/composite grafts, 93.3% for implants placed into allogenic/non-autogenous composite grafts, 95.6% for implants placed into xenograft materials alone, and only 81% for implants placed into alloplast and alloplast/xenograft materials.

The advantages of using a barrier membrane over the lateral bony window i.e., increasing the amount of vital bone formation, in sinus augmentation are well documented.25,38,39 In contradiction, Fugazzotto and Vlassis40 reported a 98.6% success rate when a bioabsorbable membrane was placed over the lateral window, whereas a similar success rate (99.2%) was observed when an external membrane was not used.

To the best of our knowledge, using an internal collagen membrane underneath the Schneiderian membrane as a routine procedure has never been investigated. In the present study, collagen membranes were routinely placed underneath the reflected Schneiderian membrane. Although most studies41-43 concluded that adequately repaired perforations have no effect on the survival of implants placed in the affected sinus, other studies reported that even if the perforation was repaired, a low quality of bone was obtained27 and that the implant survival rate at the second-stage surgery was lower in previously perforated sites compared to intact ones27,44,45 These findings may be due to potential infection from the maxillary sinus, which communicates with the outer respiratory system, as well as early resorption of the collagen membrane resulting in exposure to the sinus environment.46 The use of an internal membrane offers an additional barrier that may help to prevent passage of graft particles and bacterial contamination to and from the sinus cavity through potential small tears.47-49

CONCLUSIONS

Mineralized ground cortical bone allograft is biocompatible and osteoconductive, permitting new bone formation in sinus-augmentation procedures. The use of an internal collagen membrane on a routine basis in sinus lift procedures may have additional advantages.

ACKNOWLEDGMENT

The authors report no conflicts of interest related to this study.

REFERENCES


35. Piattelli M, Favero GA, Scavano A, Orsini G, Piattelli A. Bone reactions to anorganic bovine bone (Bio-Oss)


Correspondence: Dr. Ofer Moses, Department of Periodontology, School of Dental Medicine, Tel Aviv University, Ramat Aviv, Israel. Fax: 972-3-640-9250; e-mail: moseso@post.tau.ac.il.

Submitted February 25, 2008; accepted for publication April 30, 2008.