De Novo Bone Formation After the Sinus Lift Procedure

Albert M. Price,* Martha Nunn,† Frank G. Oppenheim,* and Thomas E. Van Dyke*

Background: The primary objective of this study is to use histomorphometric techniques to evaluate the concept that the new bone formed in the maxillary sinus lift procedure emanates from the endosteum of the sinus floor. In addition, the effect of the residual crest vertical dimension on the graft outcome and assessment of osteoclast numbers as an indirect measure of a connection between the crest and graft compartment are reported.

Methods: After grafting the maxillary sinus with irradiated allogenic bone, 37 intact, vertical bone cores with a 2.7 mm diameter were trephined at right angles to the alveolar crest. Quantitative measures were derived from a histomorphometric analysis of new bone and residual graft particles at contiguous zones along the long axis of the cores. Mean and median data were analyzed for associations with the distance from the sinus floor, dimensions of the residual crest, and other descriptive variables. A parallel series of tartrate resistant acid phosphatase-stained sections were evaluated for osteoclast counts.

Results: Mean new bone formation ranged from 24.3% to 30.2%. A statistically significant gradient of graft-particle area combined with this uniform distribution of new bone resulted in a false impression of less consolidation with the distance from the floor. There was no significant relationship between the distance from the sinus floor or dimension of the residual crest and the graft result. Mean osteoclast counts revealed a statistically significant difference ($P < 0.001$) between the residual crest and the graft compartment with increased counts in the graft.

Conclusions: Histologically, the process of new bone formation resembled a combination of de novo appositional and intramembranous ossification. The findings suggested a passive role for the graft material and implicated the ingrowth of vascular and perivascular tissues as the most logical source of osteogenic capacity. J Periodontol 2011;82:1245-1255.

KEY WORDS
Bone grafting; bone remodeling; dental implants; maxillary sinus; osteoclasts; osteogenesis.

The sinus lift bone graft has become a common method of correcting an insufficient bone dimension in the edentulous posterior maxilla for the placement of endosseous implants. Although the clinical effectiveness of the sinus lift the graft is well established,1,2 questions about the source of new bone (NB), the influence of the surrounding tissues on graft result, the contribution and fate of the graft material, and the volume of NB deemed necessary for success remain poorly defined. In the Sinus Graft Consensus Conference of 1996, restored cases with 3- to 5-year survival rates were used to measure success rates of different graft materials, either used alone or in combination. When autogenous, allogenic, and synthetic materials were reviewed, allogenic materials as a group were found to have the lowest survival rate of 85%. Frozen irradiated allogenic bone had the least success with an implant survival rate of 79.2%. In contrast, autogenous and synthetic materials were found to have implant survival rates of 88.7% and 97.5%, respectively. If graft materials were mixed, results suggested that the inclusion of an autograft was beneficial, whereas the addition of an allogenic graft might have inhibited success.1

One measure associated with a variance in outcome was the preoperative measure of the residual bone height. Based on the consensus review of available radiographic documentation for 20 failures in a subgroup of 349 implants, it was found that 65% of the failures had
residual bone height <4 mm, whereas the remaining failures had <7 mm. In contrast, cases with ≥8-mm residual height experienced zero failures.1

The consensus group felt that the minimum requirement of vital bone necessary to sustain osseointegration was thought to be 25% to 35%. They stated that this matched the normal values found in maxillary posterior cancellous bone. Histologic observations of biopsy specimens implicated the contiguous endosteum of the sinus floor and the elevated periosteum as possible sources for the newly generated bone.1

The use of trephined biopsy cores for histomorphometric evaluations has resulted in an estimate of the quantity of NB formed within the sinus lift graft area. A variety of graft materials have been examined, and the measure of NB produced has revealed a surprisingly large variation of graft vitality with a range from 8% to 60%.2-21

From a clinical perspective, the ideal result for a sinus graft would lead to a quantity of NB sufficient to provide initial surgical stability followed by resorption and remodeling of the graft material with the long-term outcome being all NB integrated with the implant.22,23 This resorption and remodeling of graft material has been generally attributed to macrophage and osteoclast activity, and yet, to our knowledge, there have been no quantitative measures of osteoclast numbers or resorption rates reported.

Identifying the source of NB, the impact of the graft material, and the effects of anatomic variation are important because they might be controlled to affect the graft outcome. A prospective human study, using irradiated allogenic vertebral bone24 as the sole graft material,‡ was designed to investigate the sinus lift bone formation and remodeling with the primary objective of testing whether the NB emanated from the sinus floor. Histomorphometric analysis of bone cores that were acquired as the first step in the osteotomy for implant placement provided quantitative data on the composition of the residual crest and graft compartment. A parallel analysis of these measures and related osteoclast numbers allowed conclusions concerning the impact of the residual crest and its vertical dimension and the relative contributions of the graft material and surrounding tissues.

**MATERIALS AND METHODS**

**Study Design and Subject Population**

The study protocol was approved by the Boston University Medical Center Institutional Review Board, and approved written consent to participate in the study was obtained from all subjects at the time of enrollment. Patients who needed a sinus lift of either maxillary sinus as part of the site preparation before placement of dental implants were enrolled from 1998 to the present. Pregnant or lactating females or patients with severe osteoporosis or uncontrolled diabetes were excluded. Before the sinus lift procedure, diagnostic templates were constructed, and the patient was referred for a preoperative computed axial tomography scan (CT). The need for sinus grafting at a site was determined by the identification of a residual bone height ≤8 mm.1 The test population consisted of eight males and 26 females with no history of bone grafts in the test area. Patients ranged in age from 43 to 84 years, with the male population ranging in age from 44 to 81 years (mean age: 67.7 years), and the female population ranging in age from 43 to 84 years (mean age: 63.2 years). Thirty-seven intact cores were obtained from the 34 patients at postgraft intervals from 6 to 18 months, with a mean of 10.4 months. Of the 37 cores, 35 cores were obtained from maxillary first molar sites, one core was obtained from the second bicusp and one core was obtained from the second molar. Core lengths ranged from 9 to 17 mm, with a mean of 12.3 mm. The residual bone height measured on preoperative CT scans ranged from 0.5 to 7.0 mm, with a mean of 3.7 mm.

**Sinus Lift Procedure and Bone Grafting**

Amoxicillin (250 mg) or clindamycin (150 mg) was prescribed four times daily at the time of surgery and continued for 10 days. The administration of local anesthesia was followed by rinsing with chlorhexidine gluconate 0.12 %.§ A full-thickness flap was reflected using 5-mm safety margins from the expected bone entry with ≥1 vertical release. The lateral bone entry was initiated 1 to 2 mm above the antral floor based on CT measures, and no attempt was made to maintain or elevate the bone window. The elevation of the sinus membrane was accomplished to a minimum vertical height of 12 mm from the crest surface. The membrane of the medial and mesial walls was elevated above the most apical aspect of the lateral window. There was no deliberate penetration or decoration of surrounding antral walls. A contoured piece of collagen dressing¶ was placed over the elevated sinus membrane to prevent the penetration of graft particles (GPs) during placement and an allogenic, irradiated, cancellous, vertebral bone graft¶ was packed into the space incrementally until the contour of the buccal wall was reestablished. The entry window was not covered with a membrane, and soft tissue closure was accomplished with 4-0 and 5-0 chromic gut sutures.# Dexamethasone (3.75 mg) was given orally after the procedure and then prescribed for a descending dose over 4 days, postoperatively. Acetaminophen

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‡ Rocky Mountain Tissue Bank, Aurora, CO.
§ Peridex Oral Rinse, 3M ESPE, 3M Center, St. Paul, MN.
¶ CollaTape, Integra LifeSciences, Plainsboro, NJ.
¶ Ethicon, Somerville, NJ.
325 mg / oxycodone 5 mg** or acetaminophen 300 mg / codeine 30 mg†† was prescribed for pain, and the postoperative follow-up and suture removal was scheduled at 2 weeks.

**Technique for Harvesting Bone Cores**

A postoperative axial CT scan was ordered at 5 to 6 months. In sites where 5- to 6-mm diameter implants were to be placed, a 3.5-mm outside diameter trephine with 2.7 mm inside diameter‡‡ was used to acquire a vertical bone core oriented at right angles to the occlusal plane as the first step in the osteotomy. The specimen was placed in 10% neutral buffered formalin, and the osteotomy was further enlarged to an appropriate size for implant placement.

**Histologic Preparations**

A process number was assigned to each core to allow for a preliminary masked evaluation. Cores were decalcified in Kristensen decalcification solution or EDTA. Paraffin-embedded cores were cut parallel to the long axis of the core at 6 μm, and ≈500 μm of the core (about one-quarter of the core diameter) was removed to achieve a horizontal section width of 2 to 2.5 mm. The next one-quarter of the core was cut into three ribbons of 25 sections and sections 1, 10, 20, 26, 36, 46, 52, 62, and 72 were mounted for hematoxylin and eosin staining. A parallel series of sections (2, 11, 21, 27, 37, 47, 53, 63, and 73) were mounted for tartrate resistant acid phosphatase (TRAP) staining.25-30 (Fig. 1).

**Histomorphometric Analysis**

Area measurements of the total field, total mineralized structure31 (bone), and total GPs were made at a magnification ×100 with image-analysis software§§ by a manual trace of respective areas on the computer screen. Results were expressed in raw values (square pixels) (Tables 1 through 3) and percentages of total field (Tables 4 and 5). In a preliminary masked test on the first 29 cores, measures of the crestal bone area (C), total bone area at the apical end of the core (NB + GPs), and GP area at the apical end of the core were determined on sections 1, 10, 20, 26, 36, 46, 52, 62, and 72. The NB area was calculated by subtraction ([NB + GPs] – GPs = NB). These bone measures were subjected to repeated-measures analysis of variance (ANOVA), and no significant difference was detected for either the bone measures in the C zone or for NB in the graft zone (P = 0.635 and P = 0.473 for C and NB, respectively), with the mean C ranging from 0.335 to 0.370 square pixels and the mean NB ranging from 0.158 to 0.186 square pixels. Based on this analysis, it was determined that final measures could be limited to sections 1, 26, and 52 which represents a 150-μm horizontal serial section interval.

**Figure 1.**

Trephine and measurements zones: outline of the core location and direction of acquisition at right angles to the crestal surface and the occlusal plane. Sections were cut at 6 μm parallel to the vertical axis of the core after about one-quarter of the diameter was removed to provide a 2 to 2.25-mm section width. Histomorphometric analysis was performed on sections 1, 26, and 51 (150-μm distance between sections). Measurement zones of the residual height and graft area are labeled C1 and C2. F1 through F5.
The final sample (N = 37) was remeasured at zone C1 and at the sinus floor zone (F1) and then in sequential contiguous vertical zones from F1 to the apical end of each core (Fig. 1). The sinus floor interface was then identified by breaking the identification code, using the preoperative measure from the respective CT scan, and confirming this in the microscope using histologic parameters and the presence of GPs. Each vertical zone was 1.5 mm in vertical dimension measured at 2 mm on center from the previous zone. In a subgroup of 10 specimens in which the residual bone height was ≥4 mm, a second vertical zone measurement of the crestal compartment (C2) was obtained.

TRAP-stained sections (2, 27, and 53) were evaluated for osteoclast number by a visual count at the same zones of C and the graft area that were previously measured for bone. Technical difficulties resulted in only 18 specimens achieving a readable stain. Area counts were done at a magnification ×200 by visual inspection, and counts for all three sections were combined for a total at each zone. Positive and negative controls for osteoclast staining were used. Typical histologies of the crestal zone, graft zone, and TRAP-stained osteoclast are shown in Figures 2A through 2C.

**Statistical Analyses**

Measurements of bone areas within microscopic fields were used to compute mean and median values for the bone area in zone C and then, in the graft compartment, the total bone area (NB + GPs), the total area of GPs, and by subtraction, the total NB ([NB + GPs] – GPs). Total area of microscopic field was standardized to 0.6901 square pixels after evaluating 30 samples. The latter was used to calculate the percentage of each component in a field. Spearman correlations were calculated for dependence between the average

### Table 1.

**Bone Measures (square pixels) for Total or Composite Bone at Each Zone (NB + GPs)**

<table>
<thead>
<tr>
<th>Zone</th>
<th>GPs n</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>37</td>
<td>0.256 ± 0.074</td>
<td>0.245</td>
<td>0.040 to 0.423</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zone 2</td>
<td>37</td>
<td>0.275 ± 0.060</td>
<td>0.266</td>
<td>0.156 to 0.413</td>
<td>0.126</td>
</tr>
<tr>
<td>Zone 3</td>
<td>36</td>
<td>0.297 ± 0.071</td>
<td>0.286</td>
<td>0.207 to 0.502</td>
<td>0.007†</td>
</tr>
<tr>
<td>Zone 4</td>
<td>25</td>
<td>0.319 ± 0.094</td>
<td>0.317</td>
<td>0.120 to 0.540</td>
<td>0.054</td>
</tr>
<tr>
<td>Zone 5</td>
<td>9</td>
<td>0.296 ± 0.058</td>
<td>0.305</td>
<td>0.181 to 0.401</td>
<td>0.041†</td>
</tr>
</tbody>
</table>

= no value available.

* For comparisons of NB at zone 1 to NB at each other zone based on the Wilcoxon signed-rank test.

† Statistical significance (P < 0.05). Repeated-measures ANOVA comparing bone + graft at zones 1 through 4 yielded a P value for within-subject comparison of 0.047.

### Table 2.

**Bone Measures (square pixels) for GP Area**

<table>
<thead>
<tr>
<th>Zone</th>
<th>GPs n</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>37</td>
<td>0.048 ± 0.037</td>
<td>0.043</td>
<td>0 to 0.137</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zone 2</td>
<td>37</td>
<td>0.076 ± 0.056</td>
<td>0.059</td>
<td>0 to 0.225</td>
<td>0.001†</td>
</tr>
<tr>
<td>Zone 3</td>
<td>36</td>
<td>0.095 ± 0.069</td>
<td>0.091</td>
<td>0.003 to 0.253</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Zone 4</td>
<td>25</td>
<td>0.118 ± 0.092</td>
<td>0.117</td>
<td>0 to 0.399</td>
<td>0.002†</td>
</tr>
<tr>
<td>Zone 5</td>
<td>9</td>
<td>0.129 ± 0.071</td>
<td>0.136</td>
<td>0.005 to 0.210</td>
<td>0.008†</td>
</tr>
</tbody>
</table>

= no value available.

* For comparisons of average GPs at zone 1 to GPs at each other zone based on the Wilcoxon signed-rank test.

† Statistical significance (P < 0.05). Repeated-measures ANOVA comparing GPs at zones 1 through 4 was conducted with a P value for within-subject comparison of 0.003.

### Table 3.

**Bone Measures (square pixels) for NB Area**

<table>
<thead>
<tr>
<th>Zone</th>
<th>NB n</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>37</td>
<td>0.208 ± 0.071</td>
<td>0.205</td>
<td>0.040 to 0.413</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zone 2</td>
<td>37</td>
<td>0.199 ± 0.062</td>
<td>0.194</td>
<td>0.105 to 0.407</td>
<td>0.338</td>
</tr>
<tr>
<td>Zone 3</td>
<td>36</td>
<td>0.201 ± 0.074</td>
<td>0.204</td>
<td>0.009 to 0.453</td>
<td>0.509</td>
</tr>
<tr>
<td>Zone 4</td>
<td>25</td>
<td>0.201 ± 0.112</td>
<td>0.169</td>
<td>0.000 to 0.438</td>
<td>0.221</td>
</tr>
<tr>
<td>Zone 5</td>
<td>9</td>
<td>0.168 ± 0.080</td>
<td>0.169</td>
<td>0.045 to 0.270</td>
<td>0.441</td>
</tr>
</tbody>
</table>

= no value available.

* For comparisons of NB at zone 1 to NB at each other zone based on the Wilcoxon signed-rank test. Repeated-measures ANOVA comparing bone as the percentage of the field at zones 1 through 4 yielded a P value for within-subject comparison of 0.740.

### Table 4.

**NB Calculated as a Percentage of Total or Composite Bone [NB/(NB + GPs)]**

<table>
<thead>
<tr>
<th>Zone</th>
<th>NB n</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>37</td>
<td>81.7 ± 13.3</td>
<td>81.9</td>
<td>56 to 100</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Zone 2</td>
<td>37</td>
<td>73.1 ± 16.7</td>
<td>74.1</td>
<td>39 to 100</td>
<td>0.001†</td>
</tr>
<tr>
<td>Zone 3</td>
<td>36</td>
<td>68.9 ± 21.4</td>
<td>68.5</td>
<td>43 to 98.8</td>
<td>0.001†</td>
</tr>
<tr>
<td>Zone 4</td>
<td>25</td>
<td>63.5 ± 25.5</td>
<td>65.9</td>
<td>0.0 to 100</td>
<td>0.007†</td>
</tr>
<tr>
<td>Zone 5</td>
<td>9</td>
<td>55.7 ± 25.6</td>
<td>55.2</td>
<td>24.9 to 97.9</td>
<td>0.008†</td>
</tr>
</tbody>
</table>

= no value available.

* For comparisons of NB at zone 1 to NB at each other zone as a percentage of the total bone based on the Wilcoxon signed-rank test.

† Statistical significance (P < 0.05). Repeated-measures ANOVA comparing the percentage of NB at zones 1 through 3 was conducted with a P value for within-subject comparison of 0.001, whereas comparing the percentage of NB at zones 1 through 4 yielded a P value for within-subject comparison of 0.007.
When the total bone area (GPs + NB) per field was measured at each contiguous zone and compared to zone F1, more total bone was found at each zone as the distance from the floor increased. Zones F3 and F5 were found to have a statistically significant greater value compared to F1 (Table 1). The percentage of total bone in each zone of C and the graft compartment was calculated and displayed in Figure 3A.

When the GP area alone was evaluated as a function of distance from the floor, significant differences between F1 and all other zones were detected with a significant increase in the GP area as the distance from the floor increased (P varied from <0.001 to 0.008; Table 2).

When NB was calculated for each zone there were no zones in which NB was significantly different from measures at F1 (Table 3). When NB was analyzed for both variables of vertical distance from the floor and the time since grafting, the only zone to have a significant relationship with time was F1 (P = 0.008), with the remaining zones having no association between distance and time (P >0.05). NB measures were dichotomized by the residual bone height (C <4 mm versus ≥4 mm), but no significant differences were found related to NB formation.

In contrast, when NB was measured as a percentage of the total bone at each zone (NB/[NB + GPs]), a significant difference between F1 and each zone was noted for every zone (P varied from 0.001 to 0.008) A gradual decrease in the percentage of NB relative to the total bone (NB + GPs) was found as the distance from the floor increased with the percentage of NB ranging from 81.7% at F1 to 55.7% at F5 (Table 4). Finally, when NB was expressed as a percentage of the total field it was found to occupy 24.3% to 30.2%. These NB measures were compared to the vital bone in the crestal zones in Table 5 and Figure 3B.

Osteoclast counts were obtained in a subset of 18 cores at the same vertical zones used to measure bone in the crestal compartment and the graft space. The mean number of osteoclasts in the graft compartment was significantly greater than the mean number in C (P <0.001) (Table 6). Within the graft compartment, osteoclasts seemed to cluster in the first zones above the floor (F1 through F3) with the number of osteoclasts in zones F2 and F3 tending to be greater than the number of osteoclasts in zone F1 (P = 0.004 and P = 0.076 comparing F2 and F3, respectively, to F1) (Table 7).

**DISCUSSION**

The primary goal of this study was to investigate the consensus statement1 that the primary source of NB in a sinus lift graft was the sinus floor. If that were true, it was hypothesized that there would be a gradual

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**Table 5.**

Percentage of Vital Bone at Each Zone (including the crestal bone) as a Percentage of the Field

<table>
<thead>
<tr>
<th>Vital Bone</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>37</td>
<td>30.2 ± 10.3</td>
<td>29.7</td>
<td>5.8 to 59.8</td>
<td>–</td>
</tr>
<tr>
<td>Zone 2</td>
<td>37</td>
<td>28.9 ± 9.1</td>
<td>28.2</td>
<td>15.2 to 59.0</td>
<td>0.338</td>
</tr>
<tr>
<td>Zone 3</td>
<td>36</td>
<td>29.3 ± 10.7</td>
<td>29.6</td>
<td>1.3 to 65.7</td>
<td>0.509</td>
</tr>
<tr>
<td>Zone 4</td>
<td>25</td>
<td>29.2 ± 16.2</td>
<td>24.4</td>
<td>0.0 to 63.4</td>
<td>0.221</td>
</tr>
<tr>
<td>Zone 5</td>
<td>9</td>
<td>24.3 ± 11.6</td>
<td>24.4</td>
<td>6.5 to 39.1</td>
<td>0.441</td>
</tr>
<tr>
<td>Average C2</td>
<td>10</td>
<td>42.9 ± 11.1</td>
<td>40.5</td>
<td>28.3 to 68.4</td>
<td>0.005†</td>
</tr>
<tr>
<td>Average C1</td>
<td>10</td>
<td>48.3 ± 13.1</td>
<td>47.7</td>
<td>28.5 to 64.8</td>
<td>0.005†</td>
</tr>
<tr>
<td>Average C2</td>
<td>37</td>
<td>51.3 ± 10.9</td>
<td>50.9</td>
<td>27.8 to 84.4</td>
<td>0.005†</td>
</tr>
</tbody>
</table>

* = no value available.

* For comparisons of NB as a percentage of the field at zone 1 to NB as a percentage of the field at each other zone based on the Wilcoxon signed-rank test.

† Indicates statistical significance (P <0.05). Repeated-measures ANOVA comparing NB as a percentage of the field at zones 1 through 4 yielded a P value for within-subject comparison of 0.740.

NB values and NB at each vertical zone for age, sex, and time. Dichotomized crestal bone (<4 and ≥4 mm) was compared to the NB at each vertical zone within the graft using two-sample t tests. ANOVA was calculated on a subset of samples (n = 10) in which C was ≥4 mm for within subject association.

Wilcoxon signed-rank tests were used to compare median values of total bone, GP area, NB area, vital bone as a percentage of the total measurement field, and (in the grafted area) vital bone as a percentage of the total bone at each zone to the same values at F1. ANOVA was conducted to compare the same measured values at each zone for within-subject comparisons.

Summary statistics for osteoclast counts at each zone in zone C and the graft area were compared with a Wilcoxon signed-rank test and a repeated-measures Freidman non-parametric test. The median number of osteoclasts in the residual bone (C1 and C2) was compared to the median number in zones F1-5.

**RESULTS**

The Spearman correlation between the age of the patient and the residual bone area in zone C or the NB in the grafted area showed no significant association (P >0.05). Likewise, when measures in C and the NB at each zone were compared by sex, there were no significant differences (P = 0.108 and P = 0.112 for average C and average NB, respectively). Thirty-seven implants of 5 to 6 mm in diameter were placed in the core acquisition sites, and there have been zero failures over a range of 4 to 12 years in function.
decrease in the NB area with the distance from the floor (i.e., a decrease in NB from F1 to F5). The results showed that the percentage of new bone formation measured in each field (Fig. 3B) was uniform in vertical and horizontal directions with the only significant association of increased NB with time seen at F1 ($P = 0.008$). This delayed increase at F1 could be explained by the fact that the elevation of the sinus membrane strips the antral floor of its periosteum leaving the cortex of the floor exposed only to the graft coagulum during initial phases of wound healing. Melcher$^{32}$ noted that the surgical elevation of the periosteum destroyed the thin basal layer and its osteogenic capacity while Price$^{33}$ in a wound healing study observed a lack of osteoclastic activity for up to 28 days on the buccal surface of the alveolar bone beneath a replaced full-thickness flap. These observations would question the capacity of either the elevated membrane or the denuded antral walls to be contributors to bone growth and/or remodeling. In the present study, it is not clear from which zone (C or F1) or when the sinus floor resorption took place, but qualitative observations suggested that it happened (Fig. 2A). The measure of remodeling reflected in the osteoclast counts suggested two independent compartments with the crestal area at a minimal maintenance level, whereas the graft area remodeled at a significantly higher level (Tables 6 and 7).

Opinions vary on whether the residual bone height affects implant survival or the graft result itself. In a retrospective study, Wheeler et al.$^{16}$ and Wheeler$^{17}$ noted that allografts had a high implant-retention rate unless the preoperative bone height was <3 mm. In contrast, a 2003 review by Wallace and Froum$^{34}$ noted that the influence of the residual bone height in the lateral-window technique was an unknown issue. In the present study, the influence of the residual bone height was evaluated as a potential variable by comparing samples with $<4$ mm to samples with $\geq 4$ mm.

The range of vertical height dimension was considerable (0.5 to 7 mm), but there was no associated difference in NB measures found in any zone of the graft compartment.

**Sinus Floor Theory: Possible Explanation**

The finding of a diminishing gradient for the measures of NB when NB was expressed as a percentage of the composite (NB + GPs) with the distance from the floor (Table 4) might explain the consensus floor theory.$^{1}$ As seen in Table 2, there was a significant gradient of increasing GP area with the distance from the sinus floor. Because NB measures (Table 3) were uniformly distributed, the NB + GP composite gave the misleading impression of less NB and less consolidation with the distance from the sinus floor (Table 4). A review of experimental designs in several studies$^{2,4,14,35}$ indicated...
that the core samples were taken in a horizontal direction above or at the graft window in the apical zone, which might have limited directional interpretations. To our knowledge, the occurrence of a graft packing gradient was not previously reported, and it might vary for different materials and/or operators. There has been considerable clinical controversy about the role of the graft material in the sinus lift procedure. The discussion has usually been limited to osteogenic capacity, osteoinductivity, or osteoconduction. The irradiated allogenic bone graft used in this study had no live cells and was not likely to have a bone morphogenetic protein inductive effect because it was not decalcified.\textsuperscript{36-38} If the conductive potential of the graft-particle surface area was a dominant factor in NB growth then the statistically significant gradient of the increasing GP area measured in this study (Table 2) should have resulted in a parallel increase in NB. In contrast, it was found that NB formation was uniform (Table 3). This could mean that the conductive properties were secondary to some other mechanism of bone formation. An alternative function of graft materials that has gained some interest in the literature is that GPs act as a space holder. Tenting experiments,\textsuperscript{39-41} in which the sinus membrane was elevated and allowed to rest on simultaneously placed implants, indicated that creating a

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**Table 6.**

Summary Statistics for Number of Average Osteoclasts for Graft Zones Versus Zone C

<table>
<thead>
<tr>
<th></th>
<th>n = 18</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>9.78 ± 4.11</td>
<td>10.67</td>
<td>1.33 to 17</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.53 ± 3.86</td>
<td>1.00</td>
<td>0 to 14</td>
<td>&lt;0.001\textsuperscript{†}</td>
<td></td>
</tr>
</tbody>
</table>

F = the graft compartment with all its zones; C = the crestal compartment and its zones; – = no value available.

* For comparisons of average osteoclasts for a graft to average osteoclasts for the residual C based on the Wilcoxon signed-rank test.

† Statistical significance (P<0.05). The repeated-measures non-parametric Friedman test comparing osteoclasts at zones 1 through 3 yielded a P value for within-subject comparison of 0.058.

---

**Figure 3.** Percentage of total bone (NB + GPs) and vital bone in each zone. **A** The percentage of total bone (NB + GPs) represented the bone available for the initial stabilization of implant. Note that a tendency for more bone was found ascending toward the apical end of specimens with zones F\textsubscript{2} through F\textsubscript{5} having values close to those found in zone C. This presented a bicortical profile and was found to be related to an increasing concentration of GPs with the distance from the floor (Table 2). **B** Percentage of vital bone in each zone represented the bone available for osseointegration. Note that NB was evenly distributed with no significant difference found with the distance from the floor. NB values at all levels within the graft area were less than values found in zone C.
De Novo

tions were combined, they suggested an independent
ences in osteoclast counts suggested independent
and vertical measures of NB in the graft area sug-
that gets coated by an appositional process including
view that NB in the sinus graft procedure emanated
the walls also contributed. A post-surgical anat-
and demineralized freeze-dried
bone mixed with demineralized freeze-dried

‡ Statistical significance (P<0.05). The repeated-measures non-parametric
Friedman test comparing osteoclasts at zones 1 through 3 yielded a
value

<table>
<thead>
<tr>
<th>Osteoclasts</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>18</td>
<td>7.067 ± 4.50</td>
<td>6.5</td>
<td>1 to 14</td>
<td>–</td>
</tr>
<tr>
<td>Zone 2</td>
<td>18</td>
<td>11.06 ± 5.29</td>
<td>9.5</td>
<td>1 to 21</td>
<td>0.004†</td>
</tr>
<tr>
<td>Zone 3</td>
<td>18</td>
<td>11.56 ± 7.22</td>
<td>11.5</td>
<td>0 to 30</td>
<td>0.076</td>
</tr>
<tr>
<td>Zone 4</td>
<td>11</td>
<td>8.64 ± 7.28</td>
<td>8</td>
<td>0 to 26</td>
<td>0.894</td>
</tr>
<tr>
<td>Zone 5</td>
<td>2</td>
<td>9.00 ± 4.24</td>
<td>9</td>
<td>6 to 12</td>
<td>–</td>
</tr>
<tr>
<td>C1</td>
<td>18</td>
<td>2.56 ± 4.48</td>
<td>0</td>
<td>0 to 14</td>
<td>0.001†</td>
</tr>
<tr>
<td>C2</td>
<td>10</td>
<td>2.20 ± 2.74</td>
<td>1</td>
<td>0 to 7</td>
<td>0.014</td>
</tr>
</tbody>
</table>

= not calculated.
* For comparisons of osteoclasts at zone 1 to osteoclasts at each other zone
based on the Wilcoxon signed-rank test.
† Friedman test comparing osteoclasts at zones 1 through 3 yielded a P value
for within-subject comparison of 0.058.

space with a blood clot alone could lead to bone de-
position on an implant.

When the crestal zones and graft zones were
compared, it was noted that mean osteoclast
counts were significantly greater in the graft com-
partment (Table 6). Qualitative observations in the
graft area revealed GPs that were covered with NB
(Figs. 2A and 2B), and at the same time, the osteo-
clast activity appeared to remodel the newly formed
bone more than the GPs (unpublished observations).
How long the GPs persist could not be answered with
the present data.

These quantitative measures showed that the
majority of NB formation within the time intervals
studied (6 to 18 months) was independent of the
direction and graft-particle surface area. The differ-
ences in osteoclast counts suggested independent
compartments of remodeling activity (Tables 6 and
7). When these findings and qualitative observa-
tions were combined, they suggested an independent
de novo bone formation that resembled intramem-
braneous ossification. This pattern of bone formation
starts as a cluster of osteogenic cells that form a core
of osteoid matrix. During this process the bone form-
ing cells become trapped as osteocytes without
formation of cement lines which is a significant differ-
ence from appositional growth. This calcified core
subsequently offers a surface for further deposition of
a conductive or appositional type42,43 (Fig. 2D).
When present, GPs may offer a competing surface
that surrounds the GPs is invaded and restructured by
the disrupted vascular channels of these same sur-
rounding tissues provide a uniform source and dis-
tribution of osteogenic cells as the healing coagulum
that surrounds the GPs is invaded and restructured by
the ingrowth of new blood vessels and their attending
pluripotent mesenchymal cells (pericytes).45-47

If we accept a 25% to 35% vitality as a target for a
graft performance, then the present series with
mean NB values of 24.3% to 30.2% (Fig. 3B; Table
5) in the graft zone compared favorably to other con-
trolled studies. The retrospective analysis of Wheeler
et al.16 and Wheeler17 of cores taken over postopera-
tive periods from 4 to 36 months measured a mean of
11.3% using sintered coral plus intraoral bone, 16.38%
NB production with coral alone, and 19.3%
using coral and iliac bone. The prospective study de-
signed by Hanisch et al.5 used grafts of anorganic bo-
vine bone mixed with demineralized freeze-dried
bone allograft and found that NB formation in the
graft area ranged from 8% ± 3% at 6 months to
20.7% ± 8% at 12 months. Suba et al.11 and Szabo
et al.,13 using an identical study design, compared
synthetic grafts of β-tricalcium phosphate (β-TCP)
to autogenous iliac grafts in the same patient. At 6
months, the measures for β-TCP grafts ranged from
32% ± 10%11 to 36% ± 6.9%13 vitality and were not
significantly different from values for iliac grafts
which yielded 34% ± 11%11 to 38% ± 7.4% vitality.13

CONCLUSIONS

The present analysis did not support the consensus
view that NB in the sinus graft procedure emanated
from the sinus floor. The uniformity in horizontal
and vertical measures of NB in the graft area sug-
gested a healing phenomenon independent of di-
rectionality. The significant difference in osteoclast
counts between the C zones and the graft area
seemed to reinforce the notion that these separate
The uniform vital bone distribution of autogenous bone (84% to 92%) and alloplasts compared favorably with the consensus numbers in this series was 100% at 4 to 12 years and residual bone height cases in which the implant was graft dependent (residual bone height <3 mm). Functional implant retention in this series was 100% at 4 to 12 years and compared favorably with the consensus numbers for autogenous bone (84% to 92%) and alloplasts (98%). The uniform vital bone distribution of 24.3% ± 11.6% to 30.2% ± 10.3% in the graft compartment satisfied ideal values proposed at the sinus consensus conference for the maintenance of osseointegration. The vertical profile of the total bone area presented by the crestal and graft compartment with its tendency to increased density in the apical zone (Fig. 3A) plus the bridging phenomena of NB between GPs (Figs. 2A and 2B) provided a bicontortical microarchitecture that facilitated the initial surgical stability. The fate of the GPs remains unresolved.

The uniformity of NB formation and the lack of association with an increased surface area of the GPs at different zones suggested that the graft’s main contribution may be space maintenance. The sinus floor endosteum possibly contributed, at some phase, to healing as noted in the results, but relative osteoclast numbers suggested a passive maintenance activity in the crestal compartment that was not associated with the active NB formation and remodeling in the graft compartment. These findings and qualitative observations, including the presence and/or absence of a cement-line formation in the NB, suggested that the NB measured in these sinus lift procedures was derived from a combination of de novo appositional and intramembraneous formation in which cells evolved from the regeneration of vascular and perivascular tissues that grew inward at a uniform rate from the entire periphery, but this proposal requires further investigation.

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Correspondence: Dr. Albert M. Price, Department of Periodontology and Oral Biology, Henry M. Goldman School of Dental Medicine, Boston University, 100 E. Newton St., Suite 217, Boston, MA 02118. Fax: 617/638-6170; e-mail: amprice@bu.edu.

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