Prospective and Randomized Evaluation of ChronOS and Bio-Oss in Human Maxillary Sinuses: Histomorphometric and Immunohistochemical Assignment for Runx 2, Vascular Endothelial Growth Factor, and Osteocalcin

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Purpose: The aim of this study was to compare ChronOS (β-tricalcium phosphate), Bio-Oss, and their addition to an autogenous bone graft in a 1:1 ratio in human maxillary sinus bone augmentation.

Materials and Methods: Thirty maxillary sinuses were divided in 5 groups: group 1 included 6 maxillary sinuses grafted with autogenous bone graft alone; group 2 included 6 maxillary sinuses grafted with ChronOS; group 3 included 6 maxillary sinuses grafted with ChronOS and autogenous bone graft in a 1:1 ratio; group 4 included 6 maxillary sinuses grafted with Bio-Oss; and group 5 included 6 maxillary sinuses grafted with Bio-Oss and autogenous bone graft in a 1:1 ratio. The number of samples for each group was determined by the statistical power test.

Results: The median areas of new bone formation in groups 1, 2, 3, 4, and 5 were 121,917.0, 83,787.0, 99,295.0, 65,717.0, and 56,230.0 μm², respectively. Statistically significant differences were found between groups 3 and 5, groups 1 and 4, and groups 1 and 5 (P < .05). The median areas of remaining biomaterial were 2,900.5, 5,291.0, 2,662.0, 56,258.5, and 64,753.5 μm² in groups 1, 2, 3, 4 and 5, respectively. Statistically significant differences occurred between groups 1 and 5, groups 3 and 5, and groups 2 and 5 (P < .05). Areas of connective tissue were 67,829.0 μm² in group 1, 97,445.9 μm² in group 2, 88,256.0 μm² in group 3, 65,501.8 μm² in group 4, and 70,203.2 μm² in group 5.

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Edentulism is a general condition that involves the elderly population, regardless of gender. Tooth loss leads to bone atrophy in the alveolar process. However, in the posterior maxillary region, it is more aggravating because of maxillary sinus pneumatization. This condition makes dental implant placement for prosthetic rehabilitation impossible; nevertheless, surgical maneuvers can increase bone height, making this treatment possible through the lateral wall approach to the maxillary sinus developed by Boyne and James.

The autogenous bone graft remains the gold standard to reconstruct areas with bone defects because of its osteogenic, osteoinduction, and osteoconduction properties. However, another surgical site is necessary for harvesting, and depending on the amount of bone graft required, there is a greater risk of morbidity to the patient. Therefore, biomaterials, such as xenografts, alloplastic grafts, and allogenic grafts, have been developed to recover maxillary sinus bone height, with promising outcomes.

One example of an osteoconductive biomaterial is β-tricalcium phosphate (βTCP). It is a microporous bone substitute with fast resorption and good results have been reported in literature; however, its predictability is unknown. ChronOS (DePuy Synthes, Paoli, CA) is a β-TCP with a homogeneous porous structure, which improves the surface area, hastens graft resorption, and facilitates biological fluid circulation and cell attachment, leading to vascular net formation and bone growth.

Another biomaterial with structural characteristics similar to bone marrow is Bio-Oss (Geistlich Pharma, Wolhusen, Switzerland). It is a xenograft whose morphology is similar to that of β-TCP, permitting the proliferation of blood vessels and bone cell migration through the interconnecting porous system. Nevertheless, studies comparing the inorganic bovine graft with β-TCP are scarce in the literature.

To understand the osteoblastic differentiation during the bone graft healing phase, it is necessary to evaluate the mediator of this process. Runx-related transcription factor 2 (Runx2) is responsible for regulating the differentiation of pluripotent mesenchymal cells in the initial period of bone repair. In addition, it regulates endochondral bone formation and vascular invasion in cartilage. Vascular endothelial growth factor (VEGF) is a growth factor responsible for the migration and proliferation of endothelial cells during the period of bone healing, allowing the vascular formation to conduct nutrients to the region of the bone graft. Osteocalcin is a protein associated with bone calcification and its expression refers to bone maturation.

The aim of this study was to compare new bone formation using ChronOS and Bio-Oss alone and added to autogenous bone graft in a 1:1 ratio with autogenous bone graft alone through histometry and immunohistochemical assessment of Runx2, VEGF, and osteocalcin after 6 months of bone healing, using ChronOS as a new bone substitute to permit dental implant placement.

The null hypothesis (H0) of this study was there would be no difference in new bone formation among the bone substitutes evaluated after 6 months of bone healing. The alternative hypothesis (H1) was there would be a difference in new bone formation among the bone substitutes evaluated after 6 months of bone healing.

### Materials and Methods

This prospective clinical study was performed at the Araçatuba Dental School of the Universidade Estadual Paulista (São Paulo, SP, Brazil), was approved by the ethical committee (protocol number 47711015.4.0000.5420), and followed the Declaration of Helsinki. All participants signed an informed consent agreement. The inclusion criteria were patients with pristine maxillary sinus bone height less than 5 mm who required bone grafting to allow dental implant placement. Patients were excluded if they had uncontrolled systemic diseases, smoked, had periodontitis, had maxillary sinus pathologies, or received radiation treatment to the head or neck. Cone-beam computed tomograms were obtained previously to evaluate all maxillary sinuses. Thirty patients with unilateral maxillary sinuses that needed grafting were invited to participate in this study and were divided into 5 groups: 6 were grafted with autogenous bone graft alone (group 1); 6 were grafted with ChronOS alone (group 2); 6 were grafted with ChronOS and autogenous bone graft in a 1:1 ratio (group 3); 6 were grafted with Bio-Oss alone (group 4); and 6 were grafted with Bio-Oss and autogenous bone graft in a 1:1 ratio (group 5). The number of the samples for each group was determined by a statistical power test based on previous results. The difference in the average to be detected was 15.1, with a standard deviation of 9.9, a significance level of 5%, and a power test of
80%, in a 1-tail hypothesis test. Randomization was performed by drawing lots to decide which patients would be grafted with each material. This was performed by a clinical assistant who was not involved in the surgeries or data evaluation.

All surgical procedures were performed under local anesthesia using 2% lidocaine with 1:100,000 epinephrine (DFL, Taquara, RJ, Brazil) by a single researcher trained in advance for this work. Cortical autogenous bone graft blocks were removed from the mandibular symphysis or retromolar region and pulverized with a bone crusher (Neodent, Curitiba, PR, Brazil) as reported by Pereira et al.33 The maxillary sinus was augmented according to the procedure described by Boyne and James.5 During the first week, all patients were administered paracetamol 500 mg (EMS, São Paulo, SP, Brazil) 4 times per day to decrease pain and amoxicillin 500 mg (EMS) 3 times per day to lower the chance of infection.

**HISTOMORPHOMETRIC ANALYSIS**

After 6 months of bone healing, biopsy samples were collected at the time of dental implant placement with a 3.0×15-mm trephine bur (MK Life, Porto Alegre, RS, Brazil) and stored in a 10% formalin solution (pH, 7) for 48 hours. The samples were washed in running water for 24 hours and decalcified in ethylenediaminetetra-acetic acid solution for 4 weeks. The solution was changed weekly. Next, samples were embedded in paraffin, sliced to a thickness of 5 μm, placed on slides, and stained with hematoxylin and eosin. Biopsy samples were evaluated by light microscopy, and images were captured using the attached digital camera (JVC TK1270 Color Video Camera; Victor Company of Japan Ltd, Tokyo, Japan) at ×12.5 magnification. Images were analyzed using the ImageJ 150c (National Institutes of Health, Bethesda, MD) in the original size. Images from 3 areas of the specimens were captured: pristine bone (2 mm above the upper side of the maxillary sinus floor) and intermediate and apical bone (2 mm below the Schneiderian membrane). Areas (square micrometers) of new bone formation, connective tissue, and remaining biomaterial were calculated, and the results of each area were added to represent the total sample. All analyses and data collection were performed by a single researcher trained in advance for this work.

**IMMUNOHISTOCHEMICAL ANALYSIS**

Primary polyclonal goat anti-human antibodies targeting VEGF, Runx2, and osteocalcin (Santa Cruz Biotechnology, Santa Cruz, CA; catalog numbers SC1881, SC8566, and SC18319, respectively) were used in immunohistochemical assays. As a secondary antibody, a biotinylated donkey anti-goat antibody (Jackson Immunoresearch Laboratories, West Grove, PA) coupled with avidin (Vector Laboratories, Burlingame, CA) was used for signal amplification, and diaminobenzidine

<table>
<thead>
<tr>
<th>Maxillary Sinus</th>
<th>Group 1 (μm²)</th>
<th>Group 2 (μm²)</th>
<th>Group 3 (μm²)</th>
<th>Group 4 (μm²)</th>
<th>Group 5 (μm²)</th>
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<td>83,787.0</td>
<td>99,205.0</td>
<td>65,717.0</td>
<td>56,230.0</td>
</tr>
</tbody>
</table>

* P < .05, statistically significant difference.
† P < .05, statistically significant difference.

Tukey multiple comparison test. For connective tissue formation, the data showed parametric results and the comparison was performed using analysis of variance, followed by the Tukey multiple comparison test (SigmaPlot 12.3; Systat Software, San Jose, CA). An a priori $P$ value less than .05 was used for all tests.

Results

HISTOMORPHOMETRIC RESULTS

The median areas of new bone formation were $121,917.0 \, \mu m^2$ in group 1, $83,787.0 \, \mu m^2$ in group 2, $99,295.0 \, \mu m^2$ in group 3, $65,717.0 \, \mu m^2$ in group 4, and $56,230.0 \, \mu m^2$ in group 5. Statistical differences occurred between groups 3 and 5, groups 1 and 4, and groups 1 and 5 ($P < .05$; Fig 1, Table 1). These results contradicted H0 and validated H1.

The median areas of the remaining biomaterial were $2,900.5 \, \mu m^2$ for group 1, $5,291.0 \, \mu m^2$ for group 2, $2,662.0 \, \mu m^2$ for group 3, $56,258.5 \, \mu m^2$ for group 4, and $64,753.5 \, \mu m^2$ for group 5. Statistical differences for remaining biomaterial occurred between groups 1 and 5, groups 3 and 5, and groups 2 and 5 ($P < .05$; Fig 2, Table 2).

The average areas of connective tissue were $67,829.0 \, \pm \, 22,984.6 \, \mu m^2$ for group 1, $97,545.9 \, \pm \, 18,983.3 \, \mu m^2$ for group 2, $88,256.0 \, \pm \, 21,820.5 \, \mu m^2$ for group 3, $65,501.8 \, \pm \, 6,297.6 \, \mu m^2$ for group 4, and $70,203.2 \, \pm \, 13,421.3 \, \mu m^2$ for group 5. Statistically significant differences occurred only between groups 2 and 4 ($P < .05$; Fig 3, Table 3).

Group 1 showed highly cellular connective tissue with the presence of vessels and mature new bone formation with a lamellar organization and osteocytes entrapped within the extracellular matrix (Fig 4). New bone formation in group 2 presented with a lamellar

STATISTICAL ANALYSIS

The Shapiro-Wilk test was performed to determine whether the outcomes had a normal distribution. No parametric data were shown for new bone formation and remaining biomaterial. The Kruskal-Wallis test was performed to compare groups, followed by the

<table>
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<th>Maxillary Sinus</th>
<th>Group 1 ($\mu m^2$)</th>
<th>Group 2 ($\mu m^2$)</th>
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<td>Median</td>
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<td>2,662.0</td>
<td>56,258.5</td>
<td>64,753.5</td>
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* $P < .05$, statistically significant difference.
† $P < .05$, statistically significant difference.
‡ $P < .05$, statistically significant difference.

organization and woven bone areas but with osteocytes in the extracellular matrix. The presence of the remaining biomaterial could be observed being reabsorbed, with new bone formation on the periphery and highly cellular connective tissue (Fig 5). In group 3, it was possible to observe particles of autogenous bone graft remaining, with empty osteocytes and lacunas and new bone formation on the periphery with lamellar organization (Fig 6). In group 4, a large number of remaining biomaterial particles were observed, with new bone formation on the periphery in intimate contact with the particles (Fig 7). Group 5 was similar to group 4, but the presence of connective tissue was more prominent (Fig 8).

IMMUNOHISTOCHEMICAL RESULTS

Immuno-labeling for Runx 2 was low (score, 1) for osteoblastic cells in groups 1, 2, 4, and 5; however, moderate (score, 2) immunostaining was found for group 3 (Figs 9A-E). For VEGF, groups 1 and 2 showed moderate (score, 2) immuno-labeling, group 5 showed low (score, 1) staining, and groups 3 and 4 showed intense (score, 3) staining (Figs 10A-E). For osteocalcin, intense staining of the bone surface (score, 3) was observed in all groups (Figs 11A-E; Table 4).

Discussion

Rates of bone graft resorption using β-TCP alone, β-TCP and autogenous bone graft (1:1), and autogenous bone graft alone were, respectively, 38.33, 43.82, and 45.75%, as reported by Gorla et al.17 In their study, they performed further analysis to evaluate new bone formation by immunohistochemical assessment and compared the materials qualitatively. Dos Santos Pereira et al34 reported more bone formation using...
an autogenous bone graft than its combination with \( \beta \)-TCP in a 1:1 ratio.

The resorption of \( \beta \)-TCP after maxillary sinus bone augmentation was similar to that of autogenous bone graft. This similarity is an advantage compared with other bone substitutes because its resorption rate indicates the need for overcorrection to obtain the expected outcome.\(^{17}\) New bone formation using pure \( \beta \)-TCP as a bone substitute has similar results to those using autogenous bone graft; however, when the mixture is in a 1:1 proportion, a delay of bone maturation is observed.\(^{34}\)

Jensen et al.\(^{35}\) reported no difference in new bone formation in maxillary sinus bone augmentation using Bio-Oss or its addition to autogenous bone graft in different proportions, and bone formation tends to improve in the long-term. In the present study, new bone formed using Bio-Oss was similar to that of its mixture with autogenous bone graft in a 1:1 ratio after 6 months of bone augmentation. However, bone formation occurred in a smaller area of the 2 groups compared with the autogenous bone graft.

Shirmohammadi et al.\(^{36}\) reported 33.13% of Bio-Oss particles remained in the maxillary sinus after
5 months of bone healing and a smaller percentage of new bone formed. The histologic feature exhibited areas of bone in contact with the biomaterial particles, no inflammatory cells, and no foreign body reaction. In the present study, the outcomes were similar for groups 4 and 5, with a notable amount of Bio-Oss particles remaining compared with the other groups evaluated.

Martinez et al.21 evaluated bone formation after maxillary sinus bone augmentation using Bio-Oss and

FIGURE 9. Histologic section showing positive immuno-labeling for runt-related transcription factor 2 (arrow) in A, group 1, B, group 2, C, group 3, D, group 4, and E, group 5 (Harris hematoxylin stain; magnification, ×25).

β-TCP. In their study, the amount of new bone formation in the maxillary sinuses grafted with β-TCP was larger and there were fewer biomaterial particles remaining. These results corroborate those of the present study; however, when autogenous bone graft was added, the mixture of β-TCP and autogenous bone in a 1:1 ratio showed more bone formation compared with the composite graft with Bio-Oss.

With the exception of group 3, the other groups evaluated in this study exhibited mature bone

**FIGURE 10.** Histologic section showing positive immuno-labeling for vascular endothelial growth factor (arrow) in A, group 1, B, group 2, C, group 3, D, group 4, and E, group 5 (Harris hematoxylin stain; magnification, ×25).

formation, areas with lamellar formation, and low (score, 1) immuno-labeling for Runx2, indicating little pre-osteoblastic activity. Despite this, osteocalcin showed intense (score, 3) staining, indicating a high degree of bone calcification. The immunostaining for VEGF presented results from moderate (score, 2) to intense (score, 3) for most groups, except for group 5, which had a low score of 1, indicating the presence of vascular proliferation. Group 3 presented a delayed maturation stage, with few osteocytes in the

**FIGURE 11.** Histologic section showing positive immuno-labeling for osteocalcin (arrow) in A, group 1, B, group 2, C, group 3, D, group 4, and E, group 5 (Harris hematoxylin stain; magnification, ×25).

extracellular matrix and moderate (score, 2) immunolabeling for Runx 2, showing a higher level of pre-osteoblastic activities.

The use of ChronOS combined with autogenous bone graft in a 1:1 ratio showed similar new bone formation as autogenous bone graft alone, indicating that the fast resorption and osteoconductivity of ChronOS make this combination a reliable bone substitute. The group grafted with Bio-Oss added to autogenous bone graft in a 1:1 ratio showed slow resorption of graft particles with discrepant outcomes compared with autogenous bone graft alone. Therefore, these results suggest that the low rates of new bone formation in the 2 groups were due to the presence of remaining Bio-Oss particles. Nonetheless, these bone substitutes have high rates of success with dental implants (range, 98.2 to 100%).

In conclusion, ChronOS combined with autogenous bone graft in a 1:1 ratio presented behavior similar to that of autogenous bone graft alone. However, the groups with Bio-Oss had histomorphometric and immuno-labeling scores for the proteins evaluated, indicating maturation of the grafted bone, thus allowing the placement of dental implants.

References


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<th>Osteocalcin</th>
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Abbreviations: Runx2, runt-related transcription factor 2; VEGF, vascular endothelial growth factor.