Histomorphometrical Analysis of Bone Formed After Maxillary Sinus Floor Augmentation by Grafting With a Combination of Autogenous Bone and Demineralized Freeze-Dried Bone Allograft or Hydroxyapatite

Rodolfo Jorge Boëck-Neto,* Mário Francisco Real Gabrielli,† Raphael Carlos Comelli Lia,‡ Elcio Marcantonio,† Jamil Awad Shibli,* and Elcio Marcantonio, Jr.*

Background: Maxillary sinus floor augmentation procedures are currently the treatment of choice when the alveolar crest of the posterior maxilla is insufficient for dental implant anchorage. This procedure aims to obtain enough bone with biomaterial association with the autogenous bone graft to create volume and allow osteoconduction. The objective of this study was to histologically and histometrically evaluate the bone formed after maxillary sinus floor augmentation by grafting with a combination of autogenous bone, from the symphyseal area mixed with DFDBA or hydroxyapatite.

Methods: Ten biopsies were taken from 10 patients 10 months after sinus floor augmentation using a combination of 50% autogenous bone plus 50% demineralized freeze-dried bone allograft (DFDBA group) or 50% autogenous bone plus 50% hydroxyapatite (HA group). Routine histological processing and staining with hematoxylin and eosin and Masson’s trichrome were performed.

Results: The histomorphometrical analysis indicated good regenerative results in both groups for the bone tissue mean in the grafted area (50.46 ± 16.29% for the DFDBA group and 46.79 ± 8.56% for the HA group). Histological evaluation revealed the presence of mature bone with compact and cancellous areas in both groups. The inflammatory infiltrate was on average non-significant and of mononuclear prevalence. Some biopsies showed blocks of the biomaterial in the medullary spaces close to the bone wall, with absence of osteogenic activity.

Conclusions: The results indicated that both DFDBA and HA associated with an autogenous bone graft were biocompatible and promoted osteoconduction, acting as a matrix for bone formation. However, both materials were still present after 10 months. *Periodontol 2002;73:266-270.

KEY WORDS
Dental implants; hydroxyapatite/therapeutic use; regeneration grafts, bone; maxillary sinus augmentation; comparison studies.

Bone availability is the key for successful placement of dental implants in the posterior maxilla, which often present loss of alveolar bone and increased maxillary sinus pneumatization, particularly when all the molars are absent.1,2

With the recognition of dental implants as a treatment option for edentulous patients, the posterior maxilla became a possible site for procedures for occlusal rehabilitation with prosthetic appliances installed over dental implants. The expanded maxillary sinus then became the basis for installing dental implants.

Boyne et al.3 defined the surgical intervention that raises the maxillary sinus floor in the posterior maxilla as the sinus lift procedure. The objective is to elevate the sinus mucosa and interpose bone grafts, with or without other osteoconduction biomaterials, between the mucosa and the sinus floor, obtaining adequate bone formation to anchor optimal length dental implants.

Bone grafts3-7 and bone graft substitutes such as demineralized freeze-dried bone allograft (DFDBA)8-9 hydroxyapatite,10-12 bioglass, calcium sulfate,13 and growth factors,14,15 alone or in combination16 are used most often.

The purpose of this study was to evaluate histomorphometrical the use of 2 bone-grafting materials, DFDBA or hydroxyapatite (HA), in combination with autogenous bone graft in sinus lifting procedures.

* Department of Periodontology, Araraquara Dental School, State University of São Paulo, Araraquara, Brazil.
† Department of Oral and Maxillofacial Surgery.
‡ Department of Pathology and Physiology.
MATERIALS AND METHODS

Study Population
Ten patients (mean age 33 ± 9.97 years) from the Department of Periodontology, Araraquara Dental School-State University of São Paulo, Brazil were selected after evaluation of their medical histories and dental examinations, including standard radiographs. A crestal bone height of 4 mm between the sinus floor and the alveolar ridge was a prerequisite. All patients gave their informed consent, which was approved by the Araraquara Dental School Ethics Committee for Human Research.

The patients were divided into 2 groups: Group 1: 5 patients who received autogenous bone graft and DFDBA§ (proportion: 1:1) for sinus grafting and Group 2: 5 patients who received autogenous bone graft and hydroxyapatite¶ (HA) (proportion: 1:1) for sinus grafting.

Sinus Floor Augmentation Technique
The sinus lift was performed using a round bur in a straight handpiece at 1,500 rpm, under copious saline solution irrigation to outline a large buccal window at the maxillary sinus lateral wall. Care was taken not to penetrate the sinus membrane. Once the outline was completed, a delicate dissection using blunt sinus curets was performed to push the sinus membrane inward and upward.

The membrane was released without any tension to provide an adequate compartment for the bone graft. Autogenous bone grafts from the symphysis area were obtained via an intraoral incision from canine to canine. Two rectangular bone blocks were harvested using a straight bur with constant irrigation, respecting the midline. The corticocancellous bone blocks were stored in saline solution until particulated with a surgical bone mill.

The graft material was then placed against the medial aspect of the compartment in the sinus cavity under meticulous condensation. The sinus buccal window was covered with a collagen membrane17-19 as previously described20 and the mucoperiosteal flap was closed over the collagen membrane with interrupted sutures.¶

The implant placements were performed 10 months after the sinus floor augmentation. At this time, bone biopsies from the alveolar crest were taken with a trephine bur# (2.0 × 8.0 mm). All dental implants installed presented good initial stability.

Histological Processing
The biopsies were harvested, fixed in formalin, and decalcified in Morse solution. Once decalcified, the original bone crest was removed and routine histologic processing and paraffin embedding were performed and 5 µm thick tissue blocks on the longitudinal plane were obtained. For each biopsy, sections were selected in the central portion of the bone cores and stained with hematoxylin and eosin (H&E) and Masson’s trichrome.

Histomorphometrical Analysis
The slides were coded so that the examiner who performed the histometric analysis was blind to the treatment. A computerized image analysis system consisting of a light microscope** coupled to a video camera†† and connected to a microcomputer ‡‡ with image analysis software§§ was used to obtain area measurements.

The central section in each specimen was selected for quantitative assessment of vital bone, marrow, and remaining particles. These measurements were expressed as a percentage of the total surface area of the bone core section.

The histological analysis was performed concurrently by 2 examiners, a periodontist and a pathologist unaware of the codes identifying the patient and treatment group. They looked for the type and the quality of tissues formed, as well as the presence of any unusual tissue reactions, such as neoplastic proliferation or necrosis.

RESULTS
Histological evaluation at 10 months revealed the presence of mature bone with compact and cancellous areas. Higher bone densities were observed in 1 DFDBA patient (Fig. 1) and 2 HA patients (Fig. 2).

---

§ Dembone, Pacific Coast Tissue Bank, Los Angeles, CA.
¶ Vicryl, Ethicon Inc., Somerville, N.J.
# 3i Implant Innovation, Palm Beach, FL.
** Diastar, Cambridge Instruments, Buffalo, NY.
†† DXC107A/107AP, Sony Electronics Inc., Tokyo, Japan.
‡‡ IBM-PC 486 DX2, IBM Corp., Rio de Janeiro, RJ, Brazil.
§§ MOCHA, Jandel Scientific, San Rafael, CA.
The cancellous bone exhibited compact areas; i.e., incremental basophilic lines mixed with interposed reversion lines. Osteoblasts demonstrating the respective osteocytes were sometimes empty. This characteristic was accentuated in the DFDBA group (Fig. 3).

The medullary spaces were ample and almost always filled with a well-vascularized connective tissue with no signs of inflammation or foreign body reaction. In the specimens with smaller bone density, the spaces filled with fatty marrow interposing areas of fibrosis that were sometimes dense. In some cases, particles of the implanted material seen as irregular vacuolate amorphous masses of basophilic tendency or as discretely eosinophilic amorphous masses were found (Fig. 4). Some of these fragments were surrounded by giant cells, characteristic of the resorption process. Sometimes the particles (hydroxylapatite) were partially or totally surrounded by fibrous tissue or integrated in the bony mass (DFDBA).

The bone formation process was well identified by the presence of osteoblasts and the Haversian system was well preserved. In one of the DFDBA cases, most of the medullary spaces were empty, containing fragments of the material. The inflammatory infiltrate was, on average, non-significant and predominantly mononuclear. In some situations, blocks of material were present close to the bony wall with absence of osteogenic activity.

Blocks of material were also seen in superficial areas, surrounded by a fibrous capsule and in some instances by inflammatory giant cells. The biopsy cores contained a mean of 50.46% and 46.79% of bone, respectively, for the DFDBA group and the HA group. The remaining particles of biomaterial for the DFDBA and HA groups were 8.24 and 6.85, respectively. The newly formed bone was in contact with the implanted material demonstrating wide interposed medullary spaces filled with marrow (Tables 1 and 2).

DISCUSSION

The posterior maxilla is a critical area for anchoring dental implants, due to bone resorption after tooth loss, decreasing height in relation to the maxillary sinus, pneumatization of the sinus, or a combination of these factors.21

When this area does not offer adequate conditions for anchoring and long-term maintenance of dental implants, there is a need to place bone grafts or bone substitutes.22-24

Maxillary sinus floor augmentation is commonly used in implant dentistry, usually combining autogenous bone grafts with other biomaterials to increase the available volume and reduce resorption. Several
There are several indications for harvesting inlay autogenous grafts from the mandible under local anesthesia. However, the amount of bone needed, especially in bilateral cases, may require the removal of graft from other areas, such as the iliac crest under general anesthesia. For this reason, viable alternatives for bone grafts substitution have been studied, for use in association with autogenous bone, decreasing the volume of autogenous bone graft needed. In this paper, the biomaterials used in association with the fresh autogenous bone showed formation of new bone that allowed primary stability for dental implants, as previously reported by several authors.

1. Both DFDBA and HA combined with autogenous bone were biocompatible and allowed osteoconduction.
2. The histomorphometrical results did not reveal any advantage for either of the materials.
3. There was a gain in bone mass in both groups and the newly-formed bone showed the usual characteristics for the area.
4. The materials were not totally resorbed after 10 months and remains were integrated with the bone.

CONCLUSIONS

ACKNOWLEDGMENTS

The authors have studied the behavior of such grafts and the quality of the newly formed bone. There are several indications for harvesting inlay autogenous grafts from the mandible under local anesthesia. However, the amount of bone needed, especially in bilateral cases, may require the removal of graft from other areas, such as the iliac crest under general anesthesia. For this reason, viable alternatives for bone grafts substitution have been studied, for use in association with autogenous bone, decreasing the volume of autogenous bone graft needed. In this paper, the biomaterials used in association with the fresh autogenous bone showed formation of new bone that allowed primary stability for dental implants, as previously reported by several authors. There is some controversy in the literature as to whether such materials allow enough bone formation by themselves for the fixation of dental implants.

The combination of autogenous bone grafts with DFDBA or hydroxyapatite in the sinus lift procedures in this study was similar to the studies of Moy et al., Wallace et al., and Wheeler et al., which demonstrated areas of bone remodeling with evidence of new bone formation.

In those reports, as well as in this study, the materials acted as osteoconductors for deposition of new bone and portions of them were incorporated into the bone. The presence of incremental lines and interposed reversion lines showed the potential of these biomaterials for new bone formation when combined with autogenous bone obtained from the symphysis, although particles of the biomaterials also became involved in fibrous tissue. The presence of giant cells in contact with the biomaterials indicated macrophagic activation, suggesting that they are resorbable, in agreement with Denissen et al. In that investigation, the authors demonstrated the formation of new bone substituting for the biomaterial.

The histological analysis showed the presence of discreet to moderate inflammatory infiltrate which suggests that the materials employed have low irritative potential and do not prevent bone regeneration. The histometrical analysis showed an average new bone formation of 50.46 ± 16.29% in the DFDBA group and 46.79 ± 8.56% in the HA group, data compatible with the results of Moy et al., who used a series of biomaterial combinations, with or without bone.

In the HA group, the cancellous bone was better organized in comparison to the DFDBA group, in agreement with Wheeler et al., who used the hydroxyapatite with or without autogenous bone graft, and obtained satisfactory results in biopsies collected after intervals from 4 to 36 months. The clinical and microscopic characteristics of the bone in this group were more appropriate for dental implant anchorage. However, this histological evaluation does not allow us to reach a conclusion.

The authors are grateful to Mrs. Ana Cláudia G.C. Miranda, laboratory technician of the Department of Periodontology, Araraquara Dental School, State University of São Paulo for histological processing. We also wish to thank the Brazilian Government (CAPES), Brasilia, DF, Brazil for financial support provided.

| Table 1. Means and Standard Deviations of Vital Bone, Remaining Biomaterial, and Marrow or Fibrous Tissue (%) in DFDBA Patients |
|---|---|---|---|
| Patient | Vital Bone | Biomaterial | Marrow and Fibrous Tissue |
| 1 | 37.52 | 4.58 | 57.9 |
| 2 | 71.32 | 10.27 | 18.41 |
| 3 | 43.23 | 13.42 | 43.45 |
| 4 | 64.39 | 7.31 | 28.30 |
| 5 | 35.86 | 5.64 | 58.50 |
| Mean ± SD | 50.46 ± 16.29 | 8.24 ± 3.60 | 41.31 ± 17.81 |

| Table 2. Means and Standard Deviations of Vital Bone, Remaining Biomaterial, and Marrow or Fibrous Tissue (%) in HA Patients |
|---|---|---|---|
| Patient | Vital Bone | Biomaterial | Marrow and Fibrous Tissue |
| 1 | 43.41 | 8.39 | 48.20 |
| 2 | 42.68 | 4.39 | 52.93 |
| 3 | 53.18 | 1.81 | 45.01 |
| 4 | 36.78 | 3.83 | 59.39 |
| 5 | 57.93 | 15.85 | 26.22 |
| Mean ± SD | 46.79 ± 8.56 | 6.85 ± 5.55 | 46.35 ± 12.48 |
**REFERENCES**


Send reprint requests to: Dr. Elicio Marcantonio Jr., Faculdade de Odontologia de Araraquara - UNESP, Rua Humaitá, 1680, 14.801-903 Araraquara, SP, Brazil. Fax: 55 16 201 6369; e-mail: elicio@foar.unesp.br.

Accepted for publication September 4, 2001.