Histomorphometric Evaluation of Human Sinus Floor Augmentation Healing Responses to Placement of Calcium Phosphate or *Ricinus communis* Polymer Associated with Autogenous Bone

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**ABSTRACT**

**Background:** Prosthetic rehabilitation of the posterior maxilla with dental implants is often difficult because of proximity to the maxillary sinus and insufficient bone height. Maxillary sinus floor augmentation procedures aim to obtain enough bone with an association between biomaterials and autogenous bone.

**Purpose:** The purpose of this study was to evaluate histomorphometrically two grafting materials (calcium phosphate and *Ricinus communis* polymer) used in maxillary sinus floor augmentation associated with autogenous bone.

**Materials and Methods:** Biopsies were taken from 10 consecutive subjects (mean age 45 years) 10 months after maxillary sinus floor augmentation. The sinus lift was performed with a mixture of autogenous bone and *R. communis* polymer or calcium phosphate in a 1:2 proportion. Routine histologic processing and staining with hematoxylin and eosin were performed.

**Results:** The histomorphometric analysis indicated satisfactory regenerative results in both groups for a mean of bone tissue in the grafted area (44.24 ± 13.79% for the calcium phosphate group and 38.77 ± 12.85% for the polymer group). Histologic evaluation revealed the presence of an inflammatory infiltrate of mononuclear prevalence that, on average, was nonsignificant. The histologic sections depicted mature bone with compact and cancellous areas in both groups.

**Conclusion:** The results indicated that both graft materials associated with the autogenous bone were biocompatible, although both were still present after 10 months.

**KEY WORDS:** calcium phosphate, dental implants/posterior maxilla, maxillary sinus floor augmentation/guided bone regeneration, *Ricinus communis*, sinus lift

Maxillary sinus floor augmentation has been used for occlusal rehabilitation with prosthetic appliances installed over dental implants in the posterior maxilla despite the fact this region often presents loss of alveolar bone and increased maxillary sinus pneumatization, particularly when all of the molars are absent.1,2 This procedure has been performed prior to or in conjunction with dental implant placement. Autogenous bone grafts are considered to be the gold standard because, owing to the lack of immunologic rejection mechanisms and the presence of stem cells and growth
factors, they have both osteoinductive and osteoconductive properties. However, one drawback to the use of autogenous bone is that the harvesting procedure requires additional surgery at the donor site, which may lead to morbidity, such as limping, paresthesia, and anesthesia, as well as residual defects. Therefore, the use of biomaterials associated with the autogenous bone graft creates volume and allows osteoconduction. Experience in this area is rapidly increasing. Bone grafts and bone graft substitutes, such as demineralized freeze-dried bone allograft (DFDBA), hydroxyapatite, calcium sulfate, and growth factors alone or in combination, are used most often.

Recently, several polymers have been used in medical applications and, for the most part, lack the rigidity, ductility, or ultimate mechanical properties required in load-bearing situations. In particular, ceramic and polymer composites are promising as bone substitute materials, especially in light of the emergence of the field of tissue engineering. This young interdisciplinary field proposes the use of synthetic or naturally derived materials to act as a scaffold for bone tissue ingrowths and organization, with subsequent dissolution of the scaffold material through the processes of biodegradation of some biomaterials and bone remodeling.

As a complement, the natural polyol derived from the castor oil plant or tropical castor bean (Ricinus communis) has emerged as a potential graft material for dental application as a bone substitute. Histologic examinations have shown fibroblastic neoformation progressively replaced by bone, with an absence of late inflammatory reaction and no signs of systemic toxic effects.

The purpose of this study was to evaluate, by histomorphometric parameters, the use of two bone grafting materials, calcium phosphate and natural polymer material (R. communis), in conjunction with autogenous bone graft in sinus lifting procedures.

MATERIALS AND METHODS

Subjects

Ten consecutive subjects (seven males and three females; mean age 45 years, range 32–59 years) from the Department of Periodontology, Dental School of Araraquara, State University of São Paulo (UNESP), Brazil, were selected after a meticulous evaluation of their medical history and dental examinations that included standard radiographs. Subjects with maxillary sinus problems, such as a recent history of acute maxillary sinusitis, were excluded. A crestal bone height of 4 mm between the sinus floor and the alveolar ridge was a prerequisite for inclusion. All subjects responded to an informed consent, which was approved by the Ethics Committee for Human Research of the Dental School of Araraquara.

The subjects were divided into two groups. The calcium phosphate group consisted of five subjects who received an autogenous bone graft and calcium phosphate (Bone Source®, Lot no. 5323 D8, Howmedica Leibinger, Inc., Dallas, TX, USA), and the R. communis polymer (Policril, Polimeros Quimicos Ltda, Araraquara, SP, Brazil) group consisted of five subjects who received an autogenous bone graft and R. communis polymer material. Both grafting materials were used with autogenous bone in a proportion in volume of two parts of graft material for one part of autogenous bone.

Maxillary Sinus Augmentation Procedure

The sinus lift technique was performed by using a round bur in a straight handpiece at 1,500 rpm under copious irrigation of saline solution 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 sinus 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 under constant saline irrigation, respecting the midline. The corticocancellous bone blocks were stored in saline solution until they were particulated with a surgical bone mill.

The graft material was then placed against the medial aspect of the compartment created in the sinus cavity under meticulous condensation. The sinus buccal window was covered with a collagen membrane (ProGuide®, Proline Biomédica, São Carlos, SP, Brazil) as previously described, and the mucoperiosteal flap was closed over the collagen membrane by interrupted sutures (Polyvicryl, Vicryl, Ethicon Inc., Somerville, NJ, USA).

The implant placements were performed 10 months after the sinus floor augmentation. At that time, bone
biopsies from alveolar crest were taken with a 2.0 × 8.0 mm trephine bur (3i Implant Innovation, Palm Beach, FL, USA). All dental implants installed presented good initial stability.

**Histologic Processing**

The block biopsies were harvested, fixed in buffered formalin, and decalcified in Morse solution. Once decalcified, routine histologic processing and paraffin embedding were done 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. The original bone crest was not evaluated histomorphometrically. These selected sections were stained with hematoxylin and eosin.

**Histomorphometric Analysis**

The slides were coded so that the examiner who performed the histomorphometric analysis was blind to treatment group. A computerized image analysis system consisting of a light microscope (Diastar, Cambridge Instruments, Buffalo, NY, USA) coupled to a video camera (DXC107A/107AP, Sony Electronics Inc., Tokyo, Japan) and connected to a microcomputer (IBM-PC 486 DX2, IBM Corp., Rio de Janeiro, RJ, Brazil) with image analysis software (MOCHA, Jandel Scientific, San Rafael, CA, USA) was used to obtain area measurements.

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

Two examiners performed the histologic analysis concurrently: one periodontist and one experienced pathologist. These examiners were both unaware of the codes identifying the patient and treatment group, and 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**

After 10 months, histologic evaluation revealed the presence of mature bone with compact and cancellous areas (Figure 1). The cancellous bone exhibits as the compact areas, incremental basophilic lines mixed with interposed reversion lines. Osteoplasts showed the respective osteocytes, and they were sometimes empty.

That characteristic is accentuated in the calcium phosphate group.

The medullary spaces are ample and almost always filled with a well-vascularized connective tissue with no signs of inflammation or foreign body reaction. The spaces are filled with fatty marrow interposing areas of fibrosis that are sometimes dense. In some cases, particles of the implanted material seen as irregular vacuolated amorphous masses of basophilic tendency or as discretely eosinophilic amorphous masses could be found (Figures 2 and 3). Giant cells, characteristic of the resorption process, involved some of these fragments. Sometimes the particles of material are partially or totally involved by fibrous tissue (polymer) or integrated into the bony mass (calcium phosphate) (Figures 4 and 5).

The bone formation process is well identified by the presence of osteoblasts, and the harvesian system is well preserved. In three cases in the calcium phosphate group, most of the medullary spaces are empty and contain fragments of split-up material. The inflammatory

![Grafted Area](image1.png)

**Figure 1** Decalcified section showing panoramic view. Note the difference between the grafted area and the “old bone” or crestal remaining bone. Polymer material can be seen in the medullary spaces and sometimes in the bone mass (hematoxylin–eosin stain; ×16 original magnification).
infiltrate is, on average, nonsignificant and of mononuclear prevalence. In some situations, the blocks of *R. communis* polymer were present close to the bony wall with the absence of osteogenic activity (Figures 6 and 7). Blocks of polymer 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 44.24 and 38.77% of bone for the calcium phosphate group and the *R. communis* polymer group, respectively (Tables 1 and 2). The remaining particles of biomaterial for calcium phosphate and *R. communis* polymer were 6.19 and 10.98%, respectively. The newly formed bone was in contact with the implanted material, showing wide interposed medullary spaces filled with marrow.

**DISCUSSION**

Limited availability and donor site morbidity represent major disadvantages of using autogenous bone, although grafting materials are known to be immunogenic and virus transmission remains a major cause of concern. Because of these problems, several grafting materials, such as synthetic calcium phosphate bone substitutes and polymers, may offer an interesting alternative for sinus floor augmentation.
The maxillary sinus floor augmentation tends to associate autogenous bone grafts with other biomaterials to increase the available volume and reduce resorption. Several authors studied the behavior of such grafts and the quality of the newly formed bone. However, there are some controversies in the literature as to whether such materials could allow enough bone formation by themselves for the fixation of dental implants.

The association of autogenous bone graft with calcium phosphate or \textit{R. communis} polymer in the sinus floor augmentation in this histologic study in humans demonstrated areas of bone remodeling with evidence of new bone formation. This histologic characteristic suggests an appropriate condition for dental implant anchorage in the posterior maxilla. Furthermore, this was the first histologic study to evaluate the healing potential and bone formation of \textit{R. communis} polymer in human sinus lifting procedures.

In this study, the materials may be considered osteoconductors for deposition of new bone, and portions of them were incorporated into the bone after 10 months. The presence of incremental lines and interposed reversion lines showed the potential of those biomaterials for new bone formation when associated with autogenous bone obtained from the symphysis, although particles of both graft materials also became involved by fibrous tissue. The presence of giant cells in contact with the polymer indicated macrophagic activation, in agreement with Lamanocarvalho and colleagues. In that article, the authors, analyzing the alveolus of rats, showed formation of new bone that replaced the polymer, although the resin polymer led to retardation in the wound healing process, revealed by a delay in bone formation in the alveolus. Kurashina and colleagues showed that the particles of calcium phosphate were involved by fibrous tissue and somewhat by new bone tissue, in agreement with our findings.

The histologic analysis showed the presence of discrete to moderate inflammatory infiltrate, which suggests that the grafting materials employed have low irritative potential. The histomorphometric analysis showed an average new bone formation of 44.24 ± 13.79% for the calcium phosphate group and 38.77 ± 12.85% for the polymer group. The calcium phosphate data differ from the results of Szabo and colleagues. These authors evaluated the use of $\beta$-tricalcium phosphate graft alone and compared it with autogenous bone in bilateral sinus floor augmentation. The authors’ findings for new bone formation range between 13.9 and 44.8% for calcium phosphate alone and 20.16 and 45.47% for autogenous bone alone. Zerbo and colleagues evaluated alveolar bone regeneration after grafting with porous tricalcium phosphate alone. The bone formed was, on average, 20% in the maxillary sinus floor and 34% for guided bone regeneration at the mandible defect. In our study, this range was 31.09 to 67.58%, suggesting that the association of calcium phosphate material with autogenous bone from the symphysis area was more effective than grafting material alone in bone formation. In addition, a previous study conducted by our group found that the association of autogenous bone with DFDBA or hydroxyapatite resulted in new bone formation. The percentage of bone formation
after maxillary sinus floor augmentation using DFDBA ranged from 35.86 to 71.32%. For hydroxyapatite, the range of bone formation was 36.78 to 57.93%. However, in that study, the authors used equal proportions of autogenous bone and graft material. In the present study, the volume of autogenous bone was decreased in 50%, although we observed a satisfactory percentage of bone formation. In addition, the new bone formation in our study allowed primary stability for all dental implants placed in both groups, as previously reported by several studies.6,9,29

In several studies, histomorphometric analysis depicts the amount of new bone formation at varying degrees.4,6,7,12,13,16,17,27 These results can be attributed to different experimental designs and variables, such as material grafting, the mixture of biomaterials with autogenous bone, and its volume and time of observation. In addition, the size or volume of the maxillary sinus and the shape of the compartment created in the sinus cavity can explain the different results observed in the literature. Although the histologic results showed a satisfactory integration between graft material and new bone, the lack of the control group using only autogenous bone may limit the power of our results.

The length of the postoperative healing period used in our study was 10 months, similar to the studies previously described.6,8,29 As reported with the other composite graft materials, prolonged healing periods may be required to allow bone maturation.6,29–32

In the calcium phosphate group, the cancellous bone was better organized in comparison with the polymer group. This may occur owing to the great physicochemical affinity between the bone matrix and calcium and phosphate ceramics.33–35 However, these results should be considered with caution, and further investigations must be conducted.
CONCLUSIONS

This study demonstrates that the use of calcium phosphate and R. communis polymer associated with autogenous bone as a graft material for sinus floor augmentation resulted in bone mass gain in both groups. Although the graft materials were biocompatible, both materials were not totally resorbed after 10 months, and the remains were integrated into the bone.

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