# Maxillary Sinus Augmentation Using Prehydrated Corticocancellous Porcine Bone: Hystomorphometric Evaluation after 6 Months

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## ABSTRACT

*Background:* Insufficient alveolar bone height often prevents the placement of standard dental implants in the posterior part of edentulous maxilla. In order to increase adequately the vertical dimension of the reabsorbed alveolar process, a sinus lift procedure is often necessary. The aim of this study was to evaluate histologic results of a prehydrated corticocancellous porcine bone used in maxillary sinus augmentation.

*Methods:* Patients (age 18–70 years) with a residual bone height requiring a maxillary sinus augmentation procedure to place dental implants were eligible for this study. All patients were treated with the same surgical technique consisting of sinus floor augmentation via a lateral approach. The space obtained by elevation of the mucosa wall was grafted with prehydrated and collagenated corticocancellous porcine bone. Biopsies were harvested 6 months after the augmentation procedures.

*Results:* Twenty-four patients were enrolled. The mean percentage of new formed bone was 43.9 ± 18.6% (range 7.5–100%), whereas the mean percentage of residual graft material was 14.2 ± 13.6% (range 0–41.9%). The new bone/residual graft material ratio in the maxillary sinuses was 3.1. The mean soft tissues percentage was 41.8 ± 22.7% (range 0–92.5%).

*Conclusion:* The present study suggested that porcine bone showed excellent osteoconductive properties and could be used successfully for sinus augmentation. Moreover, the porcine bone showed a high percentage of reabsorption after 6 months; this might be because of the presence of collagen and the porosity of the graft material.

KEY WORDS: collagenated porcine bone, reabsorption, sinus augmentation

## INTRODUCTION

Insufficient alveolar bone height can prevent placement of standard dental implants in the posterior part of edentulous maxilla. This lack of bone height may be the result of alveolar bone loss, sinus pneumatisation, or both.[1]

A sinus lift procedure can adequately increase the vertical dimension of the reabsorbed alveolar process in the posterior maxilla, thus enabling placement of implants of sufficient lengths.[2]

Various grafting materials can be used in this procedure. Autogenous bone, demineralized frozen dried bone allograft, mineralized frozen dried bone allograft xenograft, hydroxyapatite preparations, and calcium sulphate preparations have been used successfully.[3–6]

Bovine bone has been shown to have osteoconductive properties and it is well incorporated in bone tissue

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as demonstrated in both experimental and clinical study.[7] Animal studies suggested that deproteinized cancellous bovine bone granules provide an ideal scaffold for new bone formation, and the formation of bone bridges happens between and around the granules.[8]

However, some studies have pointed out that this material is not completely reabsorbable, in a sense that it will completely disappear within a year.[9] Moreover, the rate and mechanism of its reabsorption are still unclear.[10]

There is a recently studied grafting material that is a xenogenic bone substitute consisting of sterilized cortical pig bone in the form of particles with high porosity and diameter ranging from 600 to 1,000 μm.[11]

In a light microscopic and transmission electron microscopic study of human biopsies from maxillary sinus augmented with porcine bone, Orsini and colleagues indicated that this biomaterial, used alone, may promote bone formation and it can be used for maxillary sinus augmentation because it does not interfere with implant osteointegration.[12]

Nannmark and colleagues[13] have confirmed the good biocompatibility and osteoconductive properties of porcine bone. Besides, they have evaluated the addition of collagen to the bone showing that collagen may improve clinical handling. Furthermore, some other authors indicate it could play a role in the mechanism of reabsorption of particles.[14]

Although the exact influence of collagen gel on the bone tissue response to the graft is not known, it could facilitate the reabsorption of biomaterial and play a role in the improvement of its osteoconductive properties.

Therefore, the purpose of this study was to evaluate the osteoconductive and reabsorption/degradation properties of prehydrated corticocancellous porcine granules bone in sinus augmentation procedures.

## MATERIALS AND METHODS

The study involved 24 sinus augmentation procedures using delayed implant placement protocols.

### Inclusion and Exclusion Criteria

Twenty-four patients (14 males) between the age of 18 and 70 years with a bone height (residual height between 3 and 8 mm) which requires a maxillary sinus augmentation procedure to place dental implants were eligible for this study. Patients were reported as healthy and there was no requirement for routine medication.

This study was drawn up according to Declaration of Helsinki for experimentation on human subjects. Possible complications of surgical therapy were treated following the standard protocols of dental management.

An orthopantomographic radiograph and a computer tomography (CT) scan of the maxilla were taken preoperatively for each patient. Antral spaces were evaluated at 1 mm serial sections. In these patients, CT scans showed residual bone in the lateral–posterior segments of the edentulous maxilla below the floor of the maxillary sinus ranged between 3 mm and at least 8 mm of height. Furthermore, average residual bone width had to be at least 6 mm as measured by the CT scans.

### Treatment Timetable

Premedication followed the protocol suggested by Misch and Moore.[15] That is, dexamethasone 8 mg preoperatively, 6 mg after 24 hours, and 3 mg after 48 hours, as an anti-inflammatory drug. Systemic antibiotics, amoxicillin, were also administered 1 hour preoperatively (2 g) and 500 mg (*Quarter in die*) for 1 week. As an analgesic agent, Etodolac 600 mg initial dose, and 200–400 mg as needed, was also prescribed.

All the patients were treated with the same surgical technique consisting of sinus floor augmentation via a lateral approach.[16]

Once the sinus membranes were elevated to obtain the necessary volume for bone grafting, all the maxillary sinuses were grafted using 100% corticocancellous porcine bone particles (MP3®, Tecnoss, Coazze, Italy). The bony sinus windows were covered with a reabsorbable collagen membrane (Evolution®, Tecnoss, Coazze, Italy). The mucoperiosteal flaps were sutured using vertical-interrupted mattress sutures.

After 6 months a biopsy was carried out. Cylindrical bone samples were harvested with a 2.5 mm–internal diameter trephine exactly at the previous location of the lateral fractured window area in a diagonal inwards and upward direction. The ability to determine the exact location of the core area of lateral window site allows the harvesting of a pure augmented bone specimen without involving residual origin tissue.

### Tissue Processing and Analyses

Specimens were decalcified in ethylenediaminetetraacetic acid (15%) for a period of 2 weeks. Specimens were again X-rayed in order to verify the decalcification procedure. After dehydratation in graded series of ethanol, the specimens were embedded in paraffine, sectioned (3–5 μm sections), and stained with hematoxyline-eosine and modified Mallory aniline blue.

Examinations were performed in a Nikon Eclipse 80i microscope (Teknooptik AB, Huddinge, Sweden) equipped with an easy image 2000 system (Teknooptik AB) using X1.0 to X40 objectives for descriptive evaluation and morphometrical measurements.

Histomorphometric measurements were performed in order to calculate the percentages (i.e., area fraction) of mineralized bone, residual graft materials, and soft tissue components (i.e., connective tissue and/or bone marrow) 6 months after the sinus augmentation procedure.

All measurements were determined by point counting directly in the light microscope, using an optically superimposed eyepiece test square grid (distance between 6 × 6 test lines $^{1}/_{4}$ 255 mm) at a magnification of 160-fold.[17] The number of points of intersection between the test lines and the outlines of mineralized bone, bone substitute particles, and nonmineralized tissue were recorded.

### Data Analysis

The histomorphometric measurements of 24 samples of bone were obtained. The percentage of soft tissues, graft, and new bone formed were calculated for each subject and the average values were obtained.

## RESULTS

All patients were operated successfully. None of the patients complained of any pain and complications, such as sinusitis, in grafted maxillary sinuses. All the patients enrolled completed the study. All planned implants were able to be placed in the augmented sites and showed at the insertion an appropriate implant primary stability.

The histological evaluation showed that the residual graft particles were surrounded by newly formed bone which presented features of mature bone, with well-organized lamellae and numerous small osteocytic lacunae (Figure 1). In many fields, it was observed that the majority of the residual graft particles were connected by bridges to woven bone (Figures 2 and 3). All types of vessels were found both in the mineralized part and in the soft tissues. Moreover, it was also found that an active resorption of the grafted bone was taking place (Figure 4). No inflammatory cell infiltrate was present around the particles or at the bone-biomaterial interface (Figure 5).

In all 24 samples (Figure 6), the area of the new formed bone ranged from 7.5 to 100% with anaverage of 43.95 ± 18.6%, while the percentage of grafted particle area ranged from 0 to 41.9% with an average of 14.2 ± 13.6%. The soft tissues percentage was 41.8 ± 22.7% on average, and it ranged from 0 to 92.5%

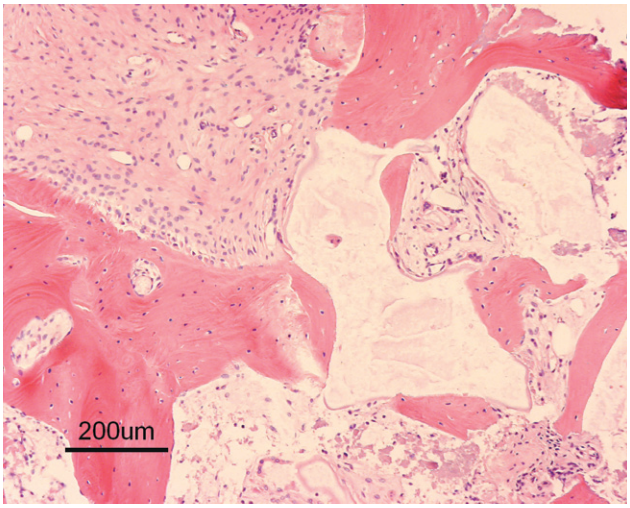


**Figure 1** Overview of biopsy 6 months after surgery. The residual graft particles were almost surrounded by newly formed bone; no signs of inflammation were observed (hematoxyline-eosine; ×10 original magnification).

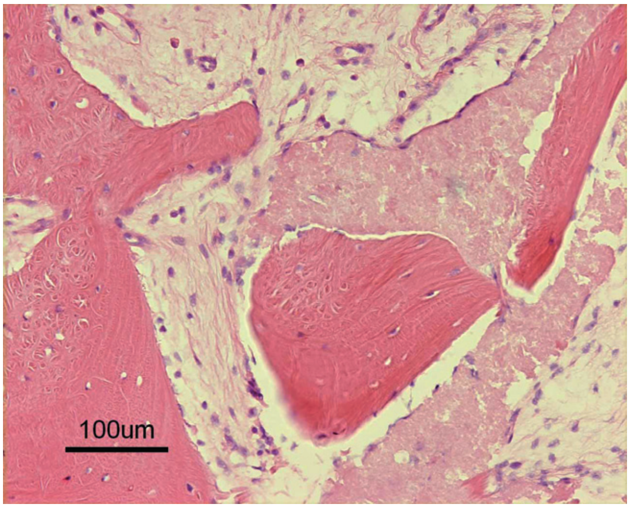


**Figure 2** Histological image 6 months after sinus lift elevation. Particles of prehydrated and collagenated porcine bone in close contact with newly formed bone (hematoxyline-eosine; ×10 original magnification).

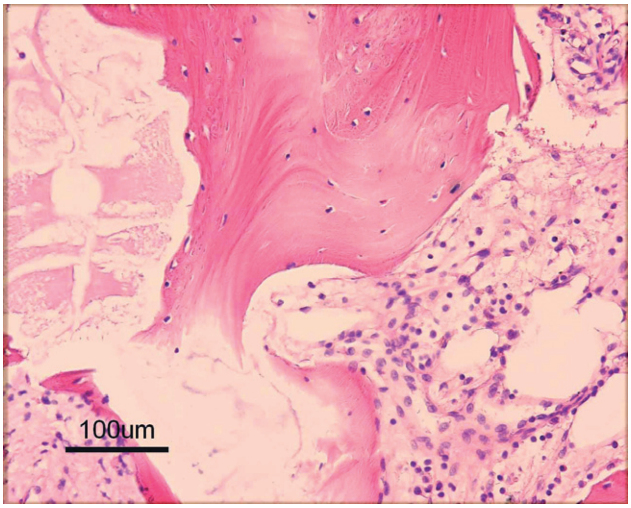


**Figure 3** Histological image 6 months after sinus lift elevation. Active resorption of porcine bone and a remodelling of bone were taking place (hematoxyline-eosine; ×10 original magnification).

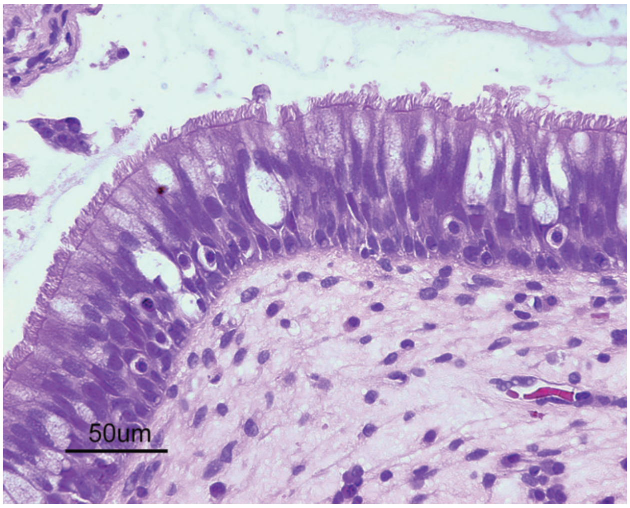


**Figure 5** Part of the sinus membrane from one biopsy. Note the unbroken epithelial lining and the ciliae. No signs of inflammation were observed (hematoxyline-eosine; ×20 original magnification).

(Table 1). The ratio between new bone and residual graft material in the augmented maxillary sinuses was 3.1.

## DISCUSSION

Bone substitutes of xenogenic origin have been frequently used as grafting materials for maxillary sinus floor augmentation procedures.[18]

Although the use of autogenous bone remains "the golden standard," other various bone substitutes have greatly evolved over the last few years in order to perform a predictable regeneration of maxillary posterior area with low morbidity for the patients.[19]

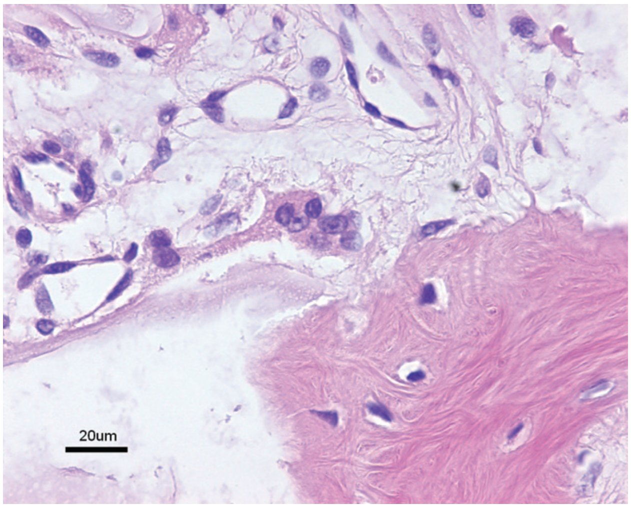


**Figure 4** Close up from Figure 1. Note the multinucleated cell showing signs of resorption of both residual graft particles and bone. The tissue was heavily vascularized (hematoxyline-eosine; ×40 original magnification).

The current study has taken under examination prehydrated corticocancellous porcine bone to evaluate the osteoconductive properties and the reabsorption rate after maxillary sinus augmentation in 24 patients.

All the patients scheduled in for this study showed a residual ridge height within 8 and 3 mm; this was considered an important clinical because the residual ridge height might be a variable to evaluate the final outcome of the procedures.[7] Therefore, in this study all patients with similar residual ridge height were treated with an identical surgical protocol. The bone core specimens were harvested 6 months after augmentation through the previous antrostomy site, rather than vertically from the alveolar ridge where native bone is present.

The histological and hystomorphometrical findings supported the idea that porcine bone had excellent osteoconductive properties. Histological results indicated that both osteogenesis and angiogenesis followed ordinary time frames, moreover, osteoblasts were observed apposing osteoid matrix directly on graft particles. In addition, the presence of multinucleated cells in resorption lacunae along the surface of porcine bone particles and the presence of bone metabolizing units within granules indicated that a remodelling/resorption processes were taking place.

The clinical success observed with porcine bone could be dependent on surface topography of the biomaterial.[20]

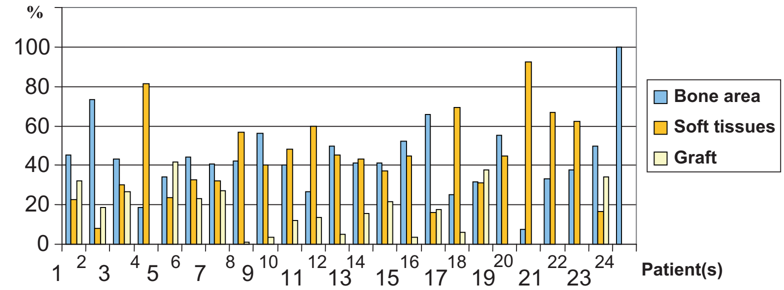


**Figure 6** Graph showing the percentage of tissues obtained in each patient after 6 months. Each patient was represented by three bars and each bar showed the tissue (bone [*blue*]; soft tissue [*yellow*]; graft material [*white*]) in the bone biopsy.

The macro and microporosity have a decisive role in osteoconduction, because both sufficient pore size and an interconnecting pore structure are required for osteoblast to grow into graft biomaterial.[21] Some authors observed that pores of 100–300 µm would be necessary for vascularisation and osteoblast migration.[22,23]

Examination of scanning electron microscopy revealed that porcine bone has microstructures similar to the human bone with a range of various pore sizes from 0.25 to 1 mm.[24]

The findings from the present study were consistent with several reports from literature where successful regenerative procedures were observed allowing dental implant placement.[25–27] Moreover, the low rate of residual graft material in our study can be considered an adjunctive advantage. The ratio between newly formed bone and the residual graft material in the maxillary sinuses after augmentation was 3.1; this confirmed a substantial resorption of the grafted material after 6 months.

Nannmark and colleagues[13] showed a relevant reabsorption of collagenated porcine bone particles after only 8 weeks in rabbits. According to the authors, it can be suggested that the presence of collagen induced adhesion of osteoclasts to the biomaterial's surface.

In addition, some authors observed that exogenous collagen I preparation can stimulate the osteoblastic phenotype probably via the natural collagen integrin interaction.[14]

It might be suggested that the presence of collagen could play a key role in starting reabsorption.[28,29] As a consequence of reabsorption activity, the presence of extracellular $Ca^{+}$, could be involved in the stimulation of osteoblasts. Yamaguchi and colleagues[30] showed that moderate high extracellular $Ca^{2+}$ is a chemotactic and proliferating signal for osteoblasts and stimulate the differentiation of preosteoblasts.

Orsini and colleagues[12] did not found any sign of resorption of porcine bone particles after 5 months in sinus augmentation procedures. Their histological results indicated only an initial reabsorption of the biomaterial as showed by an irregular outline exhibited by some peripheral regions of the particles that started to be covered by an osteoid-like fibrillar matrix after 5 months.[12] Thus, the absence of collagen in the porcine bone used in the Orsini's study could explain its incomplete reabsorption supporting our hypothesis. In addition, the fast reabsorption of porcine bone containing collagen could be considered an advantage over other biomaterials which do not contain collagen.

Bovine bone has been widely and successfully used by various authors for sinus floor elevation alone or in combination with autogenus bone.[7,31,32]

Cordaro and colleagues[33] who used bovine bone alone in sinus augmentation, indicated that the amount

**TABLE 1 Histomorphometric Data (Percentages of the Total Sample Area and Standard Deviation) 6 Months after Augmentation of 24 Maxillary Sinuses with the Use of Corticocancellous Porcine Bone**

| | Bone Area | Soft Tissues | Residual Graft |
|---|---|---|---|
| Mean (%) ± standard deviation | 43.95 ± 18.6 | 41.8 ± 22.7 | 14.2 ± 13.6 |
| Range | 7.5–100 | 0–92.5 | 0–41.9 |

of new bone after 180–240 days was 19.8 ± 7.9% on average, whereas the residual grafting material was 37.7 ± 8.5%.

Some studies on animals have demonstrated decreasing volumes of bone and osteoclastic reabsorption[34] while investigations using human biopsies with up to 6 years follow up have indicated large quantities of remaining bovine bone particles with no or few signs of reabsorption.[7,35,36]

In conclusion, findings from the present study supported the hypothesis that collagenated porcine bone has excellent osteoconductive properties and can be partially reabsorbed.

Moreover, the collagenated porcine bone allows an increase in the percentage of new bone and, at the same time, a reduction in the percentage of residual grafting material. This is very important because it is still under discussion whether the different biomaterials, such as bovine bone, will be reabsorbed with time.

However, more studies in which collagenated corticocancellous porcine bone is involved are needed before routine clinical use can be recommended.

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