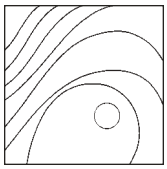
Clinical and Histologic Evaluation of Calcium Carbonate in Sinus Augmentation: A Case Series



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The aim of this case series was a clinical, histologic, and histomorphometric evaluation of calcium carbonate in sinus elevation procedures. Sinus augmentation was performed in the atrophic maxillae of 24 subjects using calcium carbonate. Six months after the regeneration procedures, 68 implants were placed and clinically followed for 1 to 5 years, depending on the placement timing. At the last implant placement procedure, 8 bone cores were harvested and processed for histology. After a 6-month healing period, sinuses grafted with calcium carbonate showed a mean vertical bone gain of 6.93 ± 0.23 mm. The histomorphometric analysis revealed 15% ± 3% residual grafted biomaterial, 28% ± 2% newly formed bone, and 57% ± 2% marrow spaces. The implant survival rate was 98.5%. It can be concluded that calcium carbonate was shown to be clinically suitable for sinus elevation procedures after 1 to 5 years of follow-up and histologically biocompatible and osteoconductive. (Int J Periodontics Restorative Dent 2014;34:e43–e49. doi: 10.11607/prd.1832)

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As a consequence of edentulism in the posterior maxilla, the vertical bone height decreases due to bone atrophy.[1] To overcome this problems, a sinus elevation procedure was first introduced by Tatum in 1986.[2] Today, this technique is still used with great success.

Several biomaterials have been proposed for use in sinus augmentation, such as autologous bone, demineralized freeze-dried bone, calcium carbonate, bioactive glass, polymer of polylactic and polyglycolide acids, bovine-derived bone and peptide, calcium sulfate, bovine deproteinized bone, and hydroxyapatite.[3] Although autologous bone shows the best results in bone regeneration, the limited availability of bone at the donor site, the need for an additional surgical procedure to harvest the bone, and postsurgical management might be considered disadvantages.[3,4] Therefore, biomaterials have been introduced to overcome these limitations.

In vivo bone regeneration in a scaffold involves the recruitment and penetration of cells from the surrounding bone tissue, as well as vascularization. High porosity is

necessary to enhance osteogenesis, and numerous studies have verified this hypothesis.[5,6] Naturally produced bioceramics, such as animal skeletons (hydroxyapatite or calcium carbonate), combine good mechanical properties with an open porosity, which makes them good candidates to be used as delivery vehicles for cells.

Due to their interconnected porous architecture, high compressive breaking stress, good biocompatibility, and resorbability, corals have been used as scaffolds for bone tissue engineering.[7,8] Natural coral exoskeleton has the best mechanical properties of the porous calcium-based ceramics, given that its interconnected porous architecture is similar to that of spongy bone. Transcortical bony defects implanted with coral become vascularised and are invaded by newly formed bone, whereas the coral is resorbed at a rate commensurate with bone formation.[7] Coral mineral (aragonite or calcite forms of calcium carbonate) has had considerable success considering its porous structure (150 to 500 µm), which is similar to that of cancellous bone, and is one of a limited number of materials that will form chemical bonds with bone and soft tissues in vivo.[3,8,9] The success of coralline implants has been linked to the presence of biologically active materials, the microstructure of the ceramic-polymer composite, and the pore architecture that provides superior strength, biocompatibility, and pore sizes large enough for cellular transport within the confines of the internal porosity. The calcium-carbonate coral-derived material (Genus porites) evaluated in this study present a chemical composition similar to bone.[10] It is constituted by more than 98% of calcium carbonate in crystal form (aragonite) and other elements (fluorine and strontium: 0.7% to 1.0%, magnesium: 0.05% to 0.2%, sodium: < 1%, potassium: < 0.03%, phosphorus: < 0.05%, water: < 0.5%, and amino acids: < 0.026%). Among all these elements, the presence of strontium is fundamental, as it can effectively promote the mineralization process.[11]

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Method and materials

Study design

Twenty-four patients (13 men and 11 women, mean age: 56.7 ± 8.1 years, range: 40 to 72 years) who required a sinus floor elevation for implant-prosthetic rehabilitation were selected for this study. The protocol of the study was approved by the Ethical Committee of the University of Guarulhos, Sao Paulo, Brazil, and all patients signed a written informed consent form. Inclusion criteria were maxillary partial edentulism involving the premolar/molar areas and the presence of a residual bone height between the sinus floor and alveolar ridge, as measured on the serial sections of the computed tomography (CT) scan in each case, ranging from 1 to 4 mm. Exclusion criteria were active infection in the sites intended for implant placement, systemic disease that could compromise osseointegration, head and neck radiation therapy, chemotherapy, uncontrolled diabetes, uncontrolled periodontal disease, and smoking.

At the initial visit, all patients underwent a clinical and occlusal examination, and periapical radiography (Fig 1), panoramic radiography, and CT were performed. Monolateral maxillary sinus floor augmentation was performed in the atrophic posterior maxillae of all subjects; in 5 of 24 patients it was performed bilaterally. A total of 29 sinuses were grafted using calcium carbonate (Biocoral, Novaxa Spa) during a 5-year period (2007 to 2012). Biocoral showed a mean porosity of 50% and was similar to cancellous bone, with an architecture composed of strongly interconnected pores of variable diameter (150 to 500 µm).[9]

Surgical procedure

All patients received antibiotics prior to surgery. The maxillary sinus augmentation was performed according to the classic technique.[2] Briefly, following a horizontal crestal incision and two vertical incisions extending beyond the mucogingival junction, a full-thickness flap was reflected to expose the maxillary sinus lateral bone wall. Under constant irrigation with saline

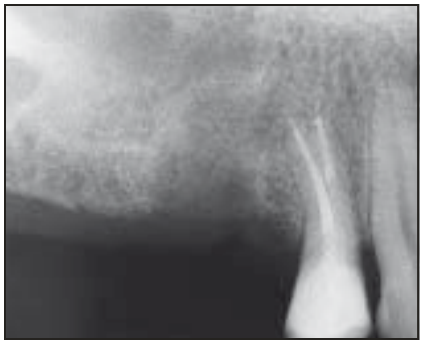

Fig 1 *Preoperative periapical radiograph of the atrophied posterior maxilla.*

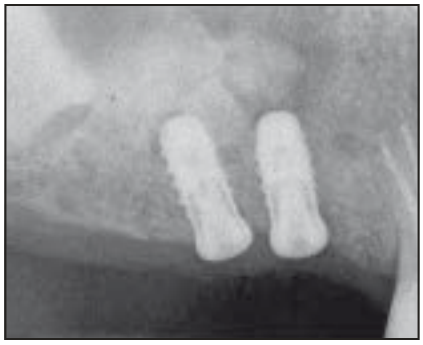

Fig 2 *Postoperative radiograph. Implants were placed 6 months after grafting.*

Table 1 Timing of implant placement in grafted sinuses after 6 months of healing

Year	Implants placed (n)
2007	15
2008	18
2009	8
2010	10
2011	8
2012	9
Total	68

solution, an osseous window of approximately 1 ± 1 cm was demarked and isolated using a round diamond-coated bur. The isolated osseous window was subsequently removed and conserved in saline solution. The sinus membrane was exposed and carefully isolated using specially designed elevators to avoid undesired perforations. In each patient, blocks of coral-derived porous hydroxyapatite were used for the augmentation procedures. To completely fill the residual gaps, granules were used. After graft material placement, the sinus augmentation procedure was completed and the previously isolated osseous window was repositioned to close the sinus lateral wall. Sutures were performed (Supramid, Novaxa Spa) to ensure complete flap closure.

After a 6-month healing period, at the reentry surgery, 68 implants (Leone, Leone Spa) were inserted (Table 1). The implants were placed under the guide of a CT template for guided bone surgery (Fig 2). Finally, 6 months after implant placement, a definitive prosthetic rehabilitation with metal-ceramic fixed prostheses was delivered. CT scans were acquired for each patient at baseline and 6 months after maxillary sinus augmentation to assess vertical bone gain. Clinical follow-ups were performed after 6 months of loading and then every year for the duration of the study.

Histologic processing

Nine bone cores were harvested using a trephine bur (2.5 × 15 mm) under copious saline solution irrigation prior to the placement of nine implants performed in 2012 (Table 1). One of nine samples was damaged during the harvesting procedure; therefore, a total of 8 specimens were histologically evaluated. All specimens were immediately fixed in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing).[12] The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycol-methacrylate resin (Technovit 7200 VLC, Kulzer). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disk at approximately 150 µm and ground to approximately 30 µm with a specially designed grinding machine

Table 2 Preoperative bone height, postoperative bone height, and vertical bone gain measured at three different levels 6 months after maxillary sinus graft augmentation

	Preoperative bone height (mm)			Postoperative bone height (mm)			Vertical bone gain (mm)*		
	Mesial	Central	Distal	Mesial	Central	Distal	Mesial	Central	Distal
Mean	3.58	2.44	1.65	10.58	9.58	8.34	7.0	7.13	6.68
SD	0.50	0.68	0.72	1.82	1.68	2.05	1.96	1.76	2.0

*Overall vertical bone gain: mean = 6.93 and SD = 0.23.

Table 3 Cumulative survival rate of implants from 0 to 60 months

Time period (mo)	Implants at the start of the interval (n)	Dropouts (n)	Implants under risk (n)	Failures (n)	Survival rate (%)	Cumulative survival rate (%)
0–6	68	0	68	1	98.5	98.5
6–12	59	0	59	0	100	98.5
12–24	51	1	50	0	100	98.5
24–36	41	2	39	0	100	98.5
36–48	33	1	32	0	100	98.5
48–60	15	0	15	0	100	98.5

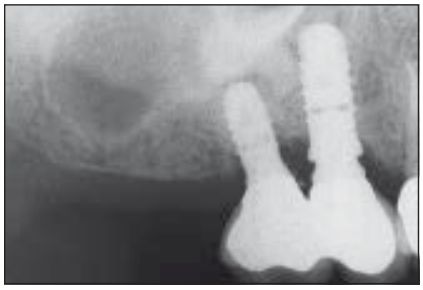

Fig 3 *Periapical radiograph 1 year after implant placement.*

(Precise 1 Automated System, Assing). The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Laborlux light microscope (Leitz). The histomorphometry was performed using the light microscope connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVC) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel). This optical system was associated with a digitizing pad (Matrix Vision) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics).

Results

In all 29 sinuses grafted with calcium carbonate, no clinical complications were observed. Measurements of vertical bone height at baseline and after 6 months of healing reveled a mean gain of 6.93 ± 0.23 mm after sinus grafting (Table 2).

All implants remained in function after 6 months of loading (Fig 3). Of 68 implants, only 1 was removed 3 months after loading due to overloading. In a follow-up period of 1 to 5 years, the implants showed a cumulative survival rate of 98.5% (Table 3).

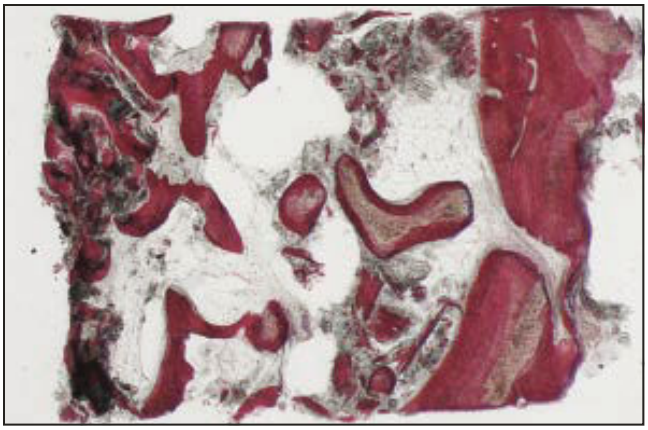

Fig 4 *Newly formed trabecular bone and residual biomaterial, which were mainly located in the apical portion of the specimens, could be observed (toluidine blue and acid fuchsin; original magnification ×12).*

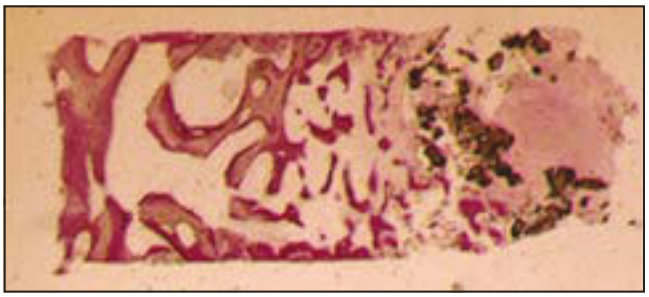

Fig 5 *Trabecular bone with wide marrow spaces can be observed and graft material remnants were present (toluidine blue and acid fuchsin; original magnification ×10).*

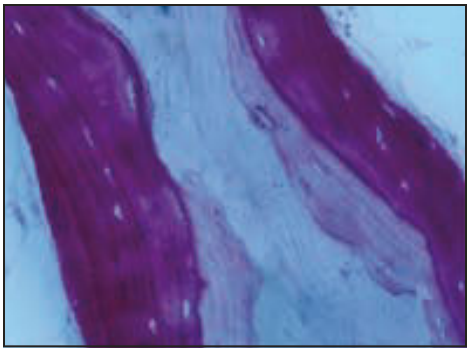

Fig 6 (left) *Newly formed bone at different stages of maturation was present (toluidine blue and acid fuchsin; original magnification ×200).*

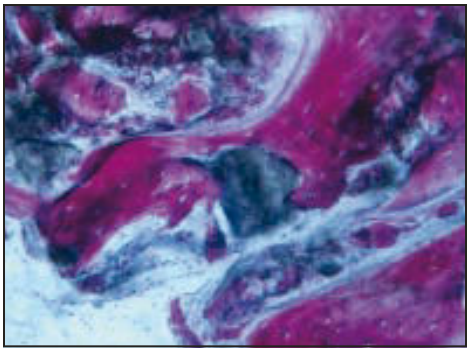

Fig 7 (right) *The residual biomaterial particles showed a small size and were partially surrounded by newly formed bone (toluidine blue and acid fuchsin; original magnification ×100).*

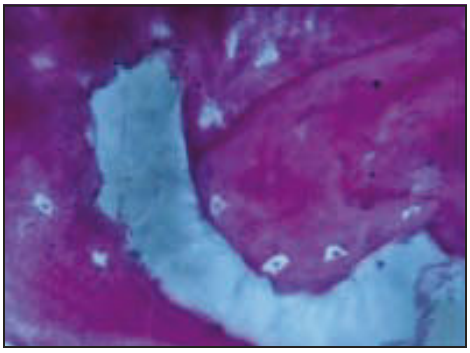

Fig 8 (left) *No gaps were present at the bone-particle interface, and newly formed bone was always in close contact with the particles (toluidine blue and acid fuchsin; original magnification ×400).*

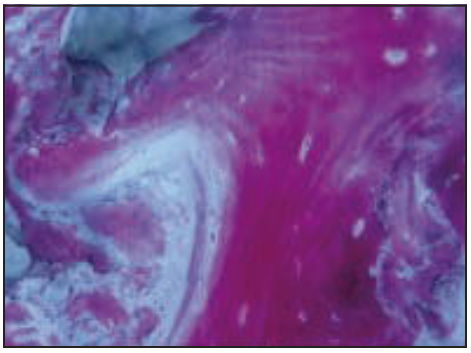

Fig 9 (right) *In some fields, it was possible to observe unmineralized osteoid matrix in close contact with residual particles (toluidine blue and acid fuchsin; original magnification ×200).*

Histologic results

Newly formed trabecular bone and residual biomaterial, which were mainly located in the apical portion of the specimens, could be observed (Figs 4 and 5). Newly formed bone at different stages of maturation was present (Fig 6); specifically, in the inner portion of the bone trabeculae was mature bone with a poor affinity for staining, and in the external portion, newly formed bone with a high affinity were detected. The different stages of maturation were lined by reversal lines. The residual biomaterial particles showed a small size and were partially surrounded by newly formed bone (Fig 7). No gaps were present at the bone-particle interface, and the bone was always in close contact with the particles (Fig 8). In some fields, it was possible to observe unmineralized osteoid matrix in close contact with residual particles (Fig 9).

The histomorphometric analysis revealed 15% ± 3% residual grafted biomaterial, 28% ± 2% newly formed bone, and 57% ± 2% marrow spaces.

Discussion

It has been demonstrated that bone ingrowth follows the scaffold's architecture: a continuous ingrowth from the outer periphery has been observed in random pore size scaffolds, scaffolds with the same sized pores and solid walls promoted discontinuous ingrowth with bone islands throughout the whole scaffold, and scaffolds with the same sized pores and porous walls resulted in both types of bone ingrowth.[13] It has been hypothesized that discontinuous bone ingrowth may result in faster healing, since bone will be forming not only from the margins but also throughout the entire space of the defect.[13]

Compared to bovine deproteinized bone, bovine-derived bone and peptide, and OsteoBiol, calcium carbonate showed the most homogeneous size distribution, a lower density than bovine deproteinized bone and bovine-derived bone and peptide, and the lowest surface area due to the large pore size of its particles.[14] Once implanted in vivo, calcium carbonate demonstrated lower trabecular bone volume and trabecular thickness than bovine deproteinized bone after 15 days of implantation and less regrown bone compared with polylactic-polyglycolic acid 30 days after surgery.[15] It has also been proved that the mineral particles of the material remain even after long implantation periods.[16] The histologic analysis performed in this study showed that newly formed bone at different stages of maturation was present in all specimens analyzed 6 months after grafting; indeed, the new bone was partially surrounded by small residual biomaterial particles.

Calcium carbonate scaffolds were used in several surgical procedures. It demonstrated the highest percentage of newly formed bone when compared with calcium sulfate and bovine deproteinized bone.[3] The material has been shown to be resorbable and to improve bone regeneration without inflammatory infiltrate or fibrous tissue around the particles. In sinus elevation procedures, it has also been demonstrated that calcium carbonate performed better than PLGA (polyglycolic-polylactic scaffold or oral bone) to allow new bone formation at the 6-month follow-up, showing a vertical bone gain of 6.47 ± 1.39 mm and 9.14 ± 1.19 mm in test (PLGA) and control (calcium carbonate) sites, respectively.[9] Similarly, in the present study, a vertical bone gain of 6.93 ± 0.23 mm was observed 6 months after the sinus elevation procedures, indicating successful new bone formation. Biocoral granules have shown good function in ridge enlargement after tooth loss, especially in the site of removal of ankylosed teeth, producing an area entirely replaced by host bone and decreasing the need for a bone graft to ensure sufficient support for dental implants.[17] In periodontal defects, a combination of guided tissue regeneration (GTR) and biocoral particles have shown better results than GTR alone[18] and biocoral particles alone[19] in terms of bone formation. Calcium carbonate was considered a beneficial bone replacement graft material for the treatment of infrabony periodontal alveolar defects; it has shown better results than open flap debridement.[20] It has also demonstrated an improvement in clinical parameters in terms of clinical attachment level, probing depth, and gingival recession after 5 years of follow-up.[21] In this case series, the implants placed in sites grafted with calcium carbonate showed a cumulative survival rate of 98.5%; the successful clinical outcomes were also corroborated by the histologic analysis.

Conclusions

Within the limitations of this study, particularly regarding the small sample size, it can be concluded that calcium carbonate was demonstrated to be clinically suitable for sinus augmentation procedures of the atrophic posterior maxilla after a 1- to 5-year time period, histologically biocompatible, and osteoconductive. Further studies are needed to assess the scaffold behavior with longer follow-up periods and a larger sample size.

Acknowledgments

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