Comparative histological results of different biomaterials used in sinus augmentation procedures: a human study at 6 months

Key words: anorganic bovine bone, biphasic calcium phosphate, calcium carbonate, phyco- gene hydroxyapatite, porcine bone, sinus augmentation procedures

Abstract
Objectives: Various grafts or combination of graft materials have been used in sinus floor augmentations, and human histological reports on their performance are available, although limited in number. Histological analysis of the regenerated tissues will provide useful information regarding the nature and amount of newly formed bone. Aim of the present study was a histological and histomorphometric evaluation, in humans, of specimens retrieved from sinuses augmented with phycogene hydroxyapatite, biphasic calcium phosphate ceramics, calcium carbonate, porcine bone and anorganic bovine bone, after a healing period of 6 months.

Materials and methods: A total of 15 patients, undergoing 30 sinus augmentation procedures with five different biomaterials, participated in this study. A total of 82 titanium dental implants were inserted in the augmented sinuses after a healing period of 6 months. A total of 60 bone cores, 2 for each augmented sinus, 12 for every biomaterial, were retrieved and all were stored immediately in 10% buffered formalin and processed to obtain thin ground sections.

Results: In all biomaterials, many grafted particles were lined and, sometimes, bridged by newly formed bone. Some biomaterials particles appeared to be partially resorbed and substituted by newly formed bone. Histomorphometry showed that, in all biomaterials, newly formed bone and residual grafted material particles represented about 30%.

Conclusions: Longer term histological and histomorphometric studies will be necessary to understand better the resorption times of all these biomaterials. The high interconnecting microporosity allowed, in all the present biomaterials, the ingrowth of newly formed bone and vessels in the pores of the partially resorbed particles. In conclusion, within the limitations of the present study, the data provided support the fact that all these biomaterials can be used, successfully, in sinus augmentation procedures.
The granules have a bone-equivalent microstructure with 65% porosity, and the specific pore volume of 1 m³/g. Every pore is limited by one layer of 35 nm. Every pore is interconnected by means of small perforations with a mean diameter of 1–3 µm. Every pore is limited by one layer of HA crystallites with a size of 25–35 nm. The granules have a bone-equivalent microarchitecture and stoichiometry.

Macroporous biphasic calcium phosphate, a mixture of 60% HA and 40% β-tricalcium phosphate (TCP), presents two types of porosity, 2/3 macropores and 1/3 micropores. The microporosity (pores smaller than 10 µm) is constituted by all the spaces between the ceramic and is necessary for the biological fluids to circulate and to promote the ionic exchange. The macroporosity (pores diameters from 300 to 600 µm) allows the ceramic to be colonized by osteogenic cells. Biphasic calcium phosphate granules are 1–2 mm in diameter and have been used as bone substitutes for sinus augmentation and other dental applications for many years (Piattelli et al. 1996). Recently, some studies have demonstrated the effectiveness of HA/TCP in sinus augmentation (Daculsi et al. 2003; Artzi et al. 2008; Cordonaro et al. 2008; Froum et al. 2008; Lee et al. 2008; Ripamonti et al. 2008; Friedman et al. 2009; Frenken et al. 2010; Covani et al. 2011).

The calcium-carbonate coral-derived material (Genus porites) analysed in the present study showed a chemical composition very similar to bone. Biocoral is constituted by more than 98% of calcium carbonate in crystal form (aragonite) and other elements [F and Sr 0.7–1%, Mg 0.05–0.2%, Na < 1%, K < 0.03%, P < 0.05%, water < 0.5%] (Ripamonti 1991; Demers et al. 2002; Wikesjo et al. 2003; Mangan et al. 2011). Among all these elements, the presence of strontium has been fundamental, as it can effectively promote mineralization processes. Biocompatible and osteoconductive, Biocoral possesses an average porosity of 50%, and it is similar to cancellous bone with an architectural composition formed by strongly interconnected pores of variable diameter (250–500 mm) (Ripamonti 1991; Demers et al. 2002; Wikesjo et al. 2003; Mangan et al. 2011).

Apatos Cortical is a collagenized cortical/cancellous bone mixture of porcine origin (Barone et al. 2005; Orsini et al. 2006; Nannmark & Sennerby et al. 2008; Barone et al. 2010; Pagliani et al. 2010; Calvo-Guirado et al. 2011; Ramirez Fernandez et al. 2011). It is constituted by carbonated nanocrystalline HA, containing organic material. Calcium-to-phosphate ratio is close to the theoretical value of 1.67 (Barone et al. 2005; Orsini et al. 2006; Nannmark & Sennerby 2008; Barone et al. 2010; Pagliani et al. 2010; Calvo-Guirado et al. 2011; Ramirez Fernandez et al. 2011).

Anorganic bovine bone (ABB) is a deproteinized stabilized bovine bone with 75–80% porosity and a crystal size of approximately 10 µm in the form of granules (Hurzeler et al. 1997; Hammerle et al. 1998; Valentini et al. 1998; Proussaefs et al. 2003; Sartori et al. 2003; Stavropoulos et al. 2003; Polyzois et al. 2007; Traini et al. 2007; Traini et al. 2008). It is constituted by a calcium-deficient carbonate apatite with a crystal size of about 10 nm and this material is identical to human bone from a chemical and physical point of view (Hurzeler et al. 1997; Hammerle et al. 1998; Valentini et al. 1998; Proussaefs et al. 2003; Sartori et al. 2003; Stavropoulos et al. 2003; Polyzois et al. 2007; Traini et al. 2007; Traini et al. 2008). ABB has a compressive strength of 35 Mpa and its porous nature (75% of the total volume) serves to greatly increase the surface area of the material (Hurzeler et al. 1997; Hammerle et al. 1998; Valentini et al. 1998; Proussaefs et al. 2003; Sartori et al. 2003; Stavropoulos et al. 2003; Polyzois et al. 2007; Traini et al. 2007; Traini et al. 2008). This increased surface area provides a substrate for an increased angiogenesis and represented a scaffold for bone formation.

Aim of the present study was a histological and histomorphometric evaluation, in humans, of specimens retrieved from sinuses augmented with phyogene HA, biphasic calcium phosphate ceramics, calcium carbonate, porcine bone and ABB, after a healing period of 6 months.

Material and methods

Fifteen healthy patients with non-contributory past medical history [six women and nine men, all non-smokers, mean age 55 years, range 51–67 years] were included in this study. The protocol of the study was approved by the Ethical Committee of the University of Guarulhos (UNIG, Sao Paulo, Brasil [CEP #15209]. All patients signed a written informed consent form. All patients were candidates for augmentation in the posterior maxilla to receive fixed restorations. The inclusion criteria were: partially edentulous patients with a bilateral loss of teeth in the maxillary premolar or molar areas with a severe alveolar atrophy and a residual alveolar ridge height between 2 and 3 mm. The exclusion criteria were: severe illness, head and neck radiation therapy, chemotherapy, uncontrolled diabetes, uncontrolled periodontal disease and smoking. After a thorough oral and physical examination, patients were scheduled for bone reconstruction procedures including sinus augmentation and implant insertion. Preoperatively, they were extensively informed concerning the surgical procedures and they were asked for their full consent.
cooperation during treatment. The patients’ mouths were rinsed with a chlorhexidine digluconate solution 0.12% for 2 min, prior to surgery. Local anaesthesia using Articaine® (Ubiestin 4% – Espe Dental AG, Seefeld, Germany) and epinephrine 1 : 100 was performed. A crestal incision was made slightly palatally, with buccal releasing incisions mesially and distally. Full thickness flaps were then elevated exposing the alveolar crest and the lateral wall of the maxillary sinus. With a ultrasonic surgery device [NSK Variosurgery Dentalica, Milano, Italy], under copious sterile saline irrigation, a window was made in the lateral sinus wall and it was rotated inwards and upwards to a horizontal position. The sinus membrane was elevated with curettes of different shapes, until it was completely detached from the lateral and inferior wall of the sinus. In all patients the sinus augmentation was bilateral. The maxillary sinuses were filled with phycogene HA (Algipore®; DENTSPLY-Friadent, Mannheim, Germany), macroporous biphasic calcium phosphate (MBCP®) (Leone, Firenze, Italy), calcium carbonate (Biocoral®, Biocoral®; Leader-Novaxa, Milan, Italy), collagenized porcine cortical/cancellous bone (Apatos Cortical, Tecnoss, Coazze, Turin, Italy), ABB (Geistlich Bio-Oss®; Geistlich, Wohlenhusen, Switzerland). In every case, 100% biomaterial was used. A total of 30 sinus augmentation procedures were performed. A total of 82 titanium dental implants were inserted in the augmented sinuses after a healing period of 6 months. A total of 60 bone cores, 2 for each augmented sinus, 12 for every biomaterial, were retrieved and all were stored immediately in 10% buffered formalin and processed to obtain thin ground sections. The specimens were processed using the Precise 1 Automated System [Assing, Rome, Italy] (Piattelli et al. 1997). The specimens were dehydrated in a graded series of ethanol rinses and embedded in a glycolmethacrylate resin (Technovit; Kulzer, Wehrheim, Germany). After polymerization the specimens were sectioned, along their longitudinal axis, with a high-precision diamond disc at about 150 μm and ground down to about 30 μm with a specially designed grinding machine. Two slides were obtained from each specimen. These slides were stained with acid fuchsin and toluidine blue and examined with transmitted light Leitz Laborlux microscope [Leitz, Wetzlar, Germany].

Histomorphometry of the percentages of newly formed bone, residual grafted material and marrow spaces was carried out using a light microscope [Laborlux S; Leitz] connected to a high resolution video camera (3CCCD, JVC KY-F55B, JVC®, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel®, Santa Clara, CA, USA). This optical system was associated with a digitizing pad [Matrix Vision GmbH, Oppenweiler, Germany] and a histometry software package with image capturing capabilities [Image-Pro Plus 4.5; Media Cybernetics Inc., Immagini & Computer Snc Milano, Italy].

Statistical analysis

The differences between the groups were statistically analysed using Dunn’s Multiple Comparisons Test, for independent samples, where statistically significant differences were accepted as $P < 0.05$. Data are presented as means ±standard deviations.

Results

Clinical results

No patient dropped out. In three sinuses regenerated, respectively, with calcium carbonate, ABB, biphasic calcium phosphate ceramics, primary stability could not be initially obtained. However, after immediately replacing the unstable implants with implants with a larger diameter, sufficient primary stability was obtained. No implant failed in all groups. Bone quality was determined as type III–IV bone during implant surgery according to the Lekholm and Zarb classification. Complications which arose after surgery consisted of two cases of nose bleeding, one in a patient in which in porcine bone had been used and the other in a patient in which phycogene HA had been used.

Radiographic analysis

Changes in bone height, after regeneration, were mainly observed in the central area of the sinuses. The average bone height at the time of surgery was $10.25 \pm 1.05$ mm, in all groups with no statistical significant differences between groups.

Histological results

In all the groups at low power magnification, it was possible to observe that many grafted particles were bridged by newly formed bone (Fig. 1). In some portions of the specimens, graft particles appeared to be lined by newly formed bone (Fig. 2). No gaps were present at the bone-particle interface and the bone was always in tight contact with the particles (Figs 3, 4). No inflammatory cells and multinucleated giant cells were present around the particles or at the interface with bone. In all the groups residual particles could be observed (Fig. 5). In the phycogene HA group most of the particles were lined by newly formed bone with large osteocyte lacunae which appeared to be always filled by osteocytes [Fig. 6]. In the porcine bone group, few peripheral osteocytic lacunae, present in the biomaterial, appeared to be filled with osteocytes [Fig. 7]. Around some particles it was

Fig. 1. The particles of phycogene hydroxyapatite were bridged by newly formed trabecular bone. Toluidine blue-basic fuchsin 40×.

Fig. 2. In some portions of the specimens, particles of biphasic calcium phosphate ceramics appeared to be surrounded by newly formed bone with a marked affinity for the staining. Toluidine blue-basic fuchsin 40×.

Fig. 3. Newly formed bone, mainly trabecular with wide marrow spaces, can be observed in tight contact with the particles of calcium carbonate. Toluidine blue-basic fuchsin 40×.
observed in the process of apposing bone directly on the particle surface. In some specimens of ABB and phycogene HA groups, the newly formed bone had grown in direct contact with the residual particles which appeared to be, in most instances, totally incorporated in the host bone (Figs 9, 10).

**Statistical comparison**

The statistical comparison of the histomorphometric data of the percentages of newly formed bone showed significant differences only between phycogene HA and calcium carbonate ($P < 0.05$). Regarding the percentages of residual graft materials significant differences were found between phycogene HA vs. calcium carbonate ($P < 0.05$), and extremely significant differences between biphasic calcium phosphate ceramics vs. porcine bone ($P < 0.001$), biphasic calcium phosphate ceramics vs. ABB ($P < 0.001$), calcium carbonate vs. porcine bone ($P < 0.001$), calcium carbonate vs. ABB ($P < 0.001$). Finally, statistical comparison of the histomorphometric percentages of marrow spaces revealed significant differences between biphasic calcium phosphate ceramics vs. porcine bone ($P < 0.05$) and calcium carbonate vs. porcine bone ($P < 0.05$) and extremely significant differences between biphasic calcium phosphate ceramics vs. ABB ($P < 0.001$) and between calcium carbonate vs. ABB ($P < 0.001$) (Tables 1 and 2).

**Discussion**

A biomaterial used in bone regeneration procedures:

1. should work as a scaffold to obtain successful integration;
2. should have an adequate pore volume, pore interconnectivity and a size of the pores large enough for vascular invasion;
3. should have mechanical characteristics similar to the tissue to be regenerated (Piattelli et al. 1999, Wallace & Froum 2003; Froum et al. 2008; Galindo-Moreno et al. 2011).

To fully assess the healing process, bone substitute materials should be evaluated histologically (Kim et al. 2009). An ideal grafting material should provide biologic stability, ensure volume maintenance and induce the formation of a high rate of vital bone and bone remodelling (Kim et al. 2009). One of the key features of synthetic graft materials is the level of macro and microporosity which play an important role in vascularization of the bone graft, which, in turn, supports the proliferation and differentiation of osteoblasts and the ingrowth of new bone into the graft (Campion et al. 2011).

Algipore® has been found to be a biocompatible, osteoconductive and resorbable biomaterial. It has been used in sinus
augmentation procedures and in the treatment of peri-implantitis. Histological evaluation has shown that a tight contact between newly formed bone and biomaterials particles, without gaps or connective fibrous tissue, was present. Porous biomaterials, such as Algipore®, act as a scaffold for tissue regeneration [Schopper et al. 2003; Ewers et al. 2004; Ewers 2005; Simunek et al. 2005]. They guide cell and vessels migration into the pores [Schopper et al. 2003; Ewers et al. 2004; Ewers 2005; Simunek et al. 2005]. Cell and vessel movement inside the pores are related to the degree of interconnection of the pores and to the pore size [Schopper et al. 2003; Ewers et al. 2004; Ewers 2005; Simunek et al. 2005].

Biphasic synthetic materials [BCP] consisting of a mixture of HA and TCP have been introduced in reconstructive implant surgery [Lindgren et al. 2009]. HA are biocompatible and do not induce a foreign body reaction and a toxic response [Frenken et al. 2010] and a significant amount of new bone has been reported with their use [Browaeys et al. 2007]. It has been claimed that an optimum balance of the stable phase of HA and the soluble phase of TCP could increase new bone formation [Artzi et al. 2008; Frenken et al. 2010]. BCP is then an osteoconductive, fully resorbable safe and biocompatible material [Artzi et al. 2008; Frenken et al. 2010]. Finally, biphasic biomimetic smart HA beta TCP matrices are endowed per se with the striking prerogative of initiating de novo bone formation by induction [Ripamonti et al. 2008]. Interconnected porosity integrates better in terms of new bone formation [Ramirez Fernandez et al. 2011]. A decrease in porosity will lead to a subsequent reduction in the flow of nutrients and flow of oxygen in a less vascular environment [Ramirez Fernandez et al. 2011].

Bioceramics, natural or synthetic, are among the most promising of all biomaterials for hard and soft tissue replacement applications. Naturally produced bioceramics, such as animal and plant skeletons [HA or calcium carbonate], can combine good mechanical properties with an open porosity. Due to their interconnected porous architecture, high compressive breaking stress, good biocompatibility and resorbability, corals have been used as scaffolds for bone tissue engineering. Coral mineral [aragonite or calcite forms of calcium carbonate] has had considerable success, considering its porous structure (150–500 μm), which is similar to that of cancellous bone and is one of the limited number of materials that will form chemical bonds with bone and soft tissues in vivo [Ripamonti 1991; Demers et al. 2002; Wikesjo et al. 2003; Mangano et al. 2011]. The pig has a genotype close to man, and xenografts of porcine origin have provided a great deal of research to assess their potential as a bone substitute [Calvo-Guirado et al. 2011]. These materials have shown to be osteoconductive, with no adverse reactions, no inflammatory infiltrate [Barone et al. 2005; Orsini et al. 2006; Nannmark & Senneryby 2008; Pagliani et al. 2010; Calvo-Guirado et al. 2011]. In animal and human studies, after a 4–6 months healing period an approximate 25–40% new bone formation was reported [Barone et al. 2005; Orsini et al. 2006; Nannmark & Senneryby 2008; Pagliani et al. 2010; Calvo-Guirado et al. 2011; Ramirez Fernandez et al. 2011]. These biomaterials have been reported to be resorbable, with clear active resorption signs of the porcine bone particles [Barone et al. 2005; Orsini et al. 2006; Nannmark & Senneryby 2008], ongoing resorption/remodelling and presence of scalloped lacunae [Pagliani et al. 2010].

**Table 1. Histomorphometric analysis**

<table>
<thead>
<tr>
<th>Material</th>
<th>Newly formed bone (%)</th>
<th>Residual grafted material (%)</th>
<th>Marrow spaces (%)</th>
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</thead>
<tbody>
<tr>
<td>Phycogene hydroxyapatite</td>
<td>33.2 ± 1.2</td>
<td>30.1 ± 0.9</td>
<td>39.3 ± 3.4</td>
</tr>
<tr>
<td>Biphasic calcium phosphate ceramics</td>
<td>30.5 ± 3.4</td>
<td>28.1 ± 0.9</td>
<td>43.6 ± 2.5</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>28.1 ± 3.9</td>
<td>27.1 ± 1</td>
<td>45.6 ± 4.5</td>
</tr>
<tr>
<td>Porcine bone</td>
<td>31.8 ± 2.9</td>
<td>33.1 ± 1.9</td>
<td>38.7 ± 2.7</td>
</tr>
<tr>
<td>Anorganic bovine bone</td>
<td>32.9 ± 0.5</td>
<td>32.8 ± 2.1</td>
<td>36.4 ± 2.3</td>
</tr>
</tbody>
</table>

**Table 2. Statistical evaluation (the statistically significant values are in bold)**

<table>
<thead>
<tr>
<th>Newly formed bone</th>
<th>Phycogene hydroxyapatite vs. biphasic calcium phosphate ceramics ns P &gt; 0.05</th>
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<tbody>
<tr>
<td>Phycogene hydroxyapatite vs. calcium carbonate</td>
<td>P &lt; 0.05</td>
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<tr>
<td>Phycogene hydroxyapatite vs. porcine bone</td>
<td>ns P &gt; 0.05</td>
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<tr>
<td>Phycogene hydroxyapatite vs. anorganic bovine bone</td>
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<td>Biphasic calcium phosphate ceramics vs. porcine bone</td>
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</tr>
<tr>
<td>Biphasic calcium phosphate ceramics vs. ABB</td>
<td>P &gt; 0.05</td>
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<tr>
<td>Calcium carbonate vs. porcine bone</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
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<td>ns P &gt; 0.05</td>
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<tr>
<td>Porcine bone vs. ABB</td>
<td>P &gt; 0.05</td>
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<tr>
<th>Residual graft materials</th>
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<tbody>
<tr>
<td>Phycogene hydroxyapatite</td>
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<tr>
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<td>ns P &gt; 0.05</td>
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<tr>
<td>Biphasic calcium phosphate ceramics vs. porcine bone</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Biphasic calcium phosphate ceramics vs. ABB</td>
<td>P &lt; 0.05</td>
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<tr>
<td>Calcium carbonate vs. porcine bone</td>
<td>P &gt; 0.05</td>
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<tr>
<td>Calcium carbonate vs. ABB</td>
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<tr>
<td>Porcine bone vs. ABB</td>
<td>P &gt; 0.05</td>
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<tr>
<th>Marrow spaces</th>
<th>Phycogene hydroxyapatite vs. biphasic calcium phosphate ceramics ns P &gt; 0.05</th>
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<tr>
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<td>Calcium carbonate vs. ABB</td>
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</tr>
<tr>
<td>Porcine bone vs. ABB</td>
<td>P &gt; 0.05</td>
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</table>
Anorganic bovine bone has been reported to undergo no or limited resorption, even if others report different results [Hurzeler et al. 1997, Hammerle et al. 1998, Valentini et al. 1998, Prussiaef et al. 2003, Sartori et al. 2003, Stavropoulos et al. 2003, Polyzois et al. 2007, Traini et al. 2007, Traini et al. 2008]. A high new bone formation has been reported with the use of ABB [Hurzeler et al. 1997, Hammerle et al. 1998, Valentini et al. 1998, Prussiaef et al. 2003, Sartori et al. 2003, Stavropoulos et al. 2003, Polyzois et al. 2007, Traini et al. 2007, Traini et al. 2008]. ABB has been shown to have osteoconductive properties, structural stability and long-term positive clinical response. Owing to its scaffolding properties and its low resorption rate, ABB may contribute significantly to prevent volume loss in grafted areas [Traini et al. 2007; Traini et al. 2008], and could withstand sinus pressure and limit bone resorption [Lambert et al. 2011]. Different opinions have been reported about the resorption capabilities of ABB: no osteoclasts on the surface of the material were observed in some studies, while, on the contrary, a resorption has been observed in other studies [Hurzeler et al. 1997, Hammerle et al. 1998; Valentini et al. 1998, Prussiaef et al. 2003, Sartori et al. 2003, Stavropoulos et al. 2003, Polyzois et al. 2007, Traini et al. 2007, Traini et al. 2008]. A high new bone formation has been reported with the use of ABB [Hammerle et al. 1998; Sartori et al. 2003, Polyzois et al. 2007; Traini et al. 2007; Traini et al. 2008]. The ultimate long-term fate of ABB is still not completely known. The slow resorption of ABB could be an advantage in that it helps in keeping the dimensions of the augmented sites, in contrast to what has been observed to happen with AB, where, in some instances, a resorption of more than 50% of the original volume of the grafted material was observed.

The results of the present study have shown that all these biomaterials can be used with success in maxillary sinus augmentation procedures showing good biocompatibility and osteoconductive properties, with osteoblastic seams forming bone directly on the biomaterial surface and with no histological signs of adverse reactions. Most of these biomaterials seem to be gradually resorbed materials, partially substituted by newly formed bone. However, after 6 months a high quantity of the biomaterial particles was still present. Longer term histological and histomorphometric studies will be necessary to understand better the resorption times of all these biomaterials. The high interconnecting microporosity allowed, in all the present biomaterials, the ingrowth of newly formed bone and vessels in the pores of the partially resorbed particles.

In conclusion, within the limitations of the present study, the present data support the fact that all these biomaterials can be used, successfully, in sinus augmentation procedures.

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References


