Evaluation of sinus floor elevation using a composite bone graft mixture

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Date:
Accepted 15 May 2006

To cite this article:
doi: 10.1111/j.1600-0501.2007.01337.x

Key words: autogenous bone, bone regeneration, bovine hydroxyapatite, implants, platelet-rich plasma, PRP, sinus lift

Abstract
Background: The performance of implant surgery in the posterior maxilla often poses a challenge due to insufficient available bone. Sinus floor elevation was developed to increase needed vertical height to overcome this problem. The present study described and reported a simple, safe and predictable bone graft mixture for the sinus lifting procedure.

Material and methods: Seventy patients were recruited for this study and underwent a sinus lift procedure. All sites were treated with a composite graft of cortical autogenous bone, bovine bone and platelet-rich plasma (PRP). A total of 263 implants (171 Astra Tech and 92 Microdent) were placed either simultaneously or delayed. All sites were clinically and radiographically evaluated 24 months after their prosthetic loading. Biopsy samples were taken from 16 delayed implant placement sites at the time of their implant placement.

Results: A 100% implant success rate was found after 24 months of functioning. Only two Microdent implants failed before loading, which translates to a 99% overall implant success rate. No statistically significant differences were found between simultaneous and delayed implant placement. Image processing revealed 34.6 ± 6.34% vital bone, 49.6 ± 6.04% connective tissue and 16.4 ± 3.23% remaining Bio-Oss particles. However, the histomorphometric analysis showed that the bovine bone was incorporated into new bone formation.

Conclusion: The results showed that a composite graft comprised of cortical autogenous bone, bovine bone and PRP mixture can be successfully used for sinus augmentation.

Tooth loss in the posterior maxilla results in a rapid resorption of both horizontal and vertical alveolar bone due to lack of intraosseous stimulation by periodontal ligament fibers [Bays 1986]. In addition, the absence of upper molars leads to increased osteoclast activity in Schneider’s membrane, causing pneumatization of the sinus by resorbing bone within a few months.

It is widely acknowledged that the best therapeutic option for replacing absent teeth is the placement of osseointegrated implants [Van Steenberghe 2000]. However, their placement in the posterior maxilla frequently poses a challenge because of the small height of residual bone and the supposedly ‘poor quality’ of bone in this area [Davies 2003]. One cause of failure of most implants placed in these areas without sinus lifting is not so much the ‘quality’ of type IV bone but rather the use of implants that are too short to resist the strong occlusal forces exerted in this area [Zinner & Small 2004]. If a surgical sinus lift technique is not applied before implant treatment, there is also a risk of perforation.
of the sinus membrane that may result in sinusitis, possible implant migration to the maxillary sinus and other complications [Kamada et al. 2003, Raghoebar & Vissink 2003; Galindo et al. 2003].

In the 1980s, Boyne & James [1980] and Tatum [1986] described techniques for bone grafting of the maxillary sinus with the aim of obtaining more bone and increasing the likelihood of successful implant placement. Since then, various modifications of the technique and different filling materials have been proposed, aimed at reducing complications and increasing the success rate.

The rationale for using a composite bone graft that includes cortical autogenous bone, bovine bone and platelet-rich plasma [PRP] is explained as follows: Autologous bone grafts obtained from the patient are the most widely used bone graft [Daelemans et al. 1997]. These can be procured either intraorally [from the mandible [Cordaro 2003], the tuberosity itself [Pacifici et al. 2003], extraorally [from the iliac crest [Triplette & Schow 1996; Lorenzetti et al. 1998; Timmenenga et al. 2003], calota [Iizuka et al. 2004] or even from the tibia [Herford et al. 2003]]. The autograft has been regarded as the gold standard for sinus floor elevation [Daelemans et al. 1997; Cordaro 2003] because it contains osteogenic, osteoinductive, osteoconductive properties, a high number of viable cells and is rich in growth factors [such as PDGF and TGF-β] [Mundy et al. 1995; Khan et al. 2000]. The viable cells consist of osteoblasts, undifferentiated mesenchymal cells, monocytes and osteoclast precursor cells [Takahari et al. 2002], and participate in the remodeling and formation of de novo bone [Martin & Sims 2005]. The enriched growth factors promote proper bone healing. Nevertheless, due to its limited availability and potential donor site morbidity, bone substitutes such as demineralized freeze-dried bone allograft [DFDBA] [Piattelli et al. 1996; Paul et al. 2001], bovine bone [Maizara et al. 2000], resorbable and non-resorbable hydroxyapatite [HA] [Moy et al. 1993; Karabuda et al. 2001; Haas et al. 2003], tricalcium phosphate [Scher et al. 1999; Zerbo et al. 2003] and coraline derivatives composed of phosphate and calcium carbonate [Velich et al. 2004] have been developed and used.

Bovine anorganic bone [Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland] was a popular bone graft for this procedure [Hürzeler et al. 1997; Piattelli et al. 1999; Valentini et al. 2000]. It is a biologically safe material but also remains long enough to permit slow apposition of de novo bone formation. It has been widely used and associated with high clinical success rates [Carmagnola et al. 2000, 2002]. The use of bovine bone in combination with autologous bone offers many additional advantages. First, it allows the volume of the graft to be doubled, avoiding the need to harvest large amounts of autologous bone. Second, the osteoconductive properties of bovine bone act as a scaffold that is essential for bone remodeling [Davies 1996]. Third, bovine bone is a calcium-deficient carbonate apatite with a crystal size of approximately 10 nm [Paul et al. 1993]. Thus, the surface area of each graft particle is considerably greater than that of porous bioceramics, making its resorption considerably slower. This could maintain the space longer, which is another prerequisite for the bone augmentation [Wang & Boyapati 2006]. Lastly, its modulus of elasticity is similar to that of natural bone [Rueger 1992] to ensure a proper uneventful healing. It is because of these properties that we chose this bone graft.

Recently, PRP was advocated for use in sinus floor elevation [Philippart et al. 2005] due to its high concentration of growth factors [platelet-derived growth factors, insulin-like growth factors as well as vascular endothelium growth factors and transforming growth factor-β]. Marx et al. reported a 1.62–2.16-fold greater bone maturation of grafts mixed with PRP and a higher bone density (74 ± 11% vs. 55.1 ± 1%) at sites where PRP was added in comparison with grafts and sites, respectively, without PRP addition. Many authors have reported positive sinus lift outcomes after using PRP mixed with bone substitute, whether autogenous, allogenic or alloplastic [Kassolis et al. 2000; Rosenberg & Torosian 2000; Lozada et al. 2001; Fürst et al. 2003, Maiorana et al. 2003, Rodríguez et al. 2003]. Besides the above-mentioned properties, PRP has an important adhesive capacity via its hemostatic capacity of fibrin [Rousou et al. 1984; Yoshida et al. 2000; Vaiman et al. 2005]. This facilitates handling of the bone graft mixture [Vachiramon et al. 2002].

Hence, it was the purpose of this study to evaluate clinically, radiographically, as well as histomorphometrically the efficacy of this composite graft (autogenous bone, bovine HA and PRP) during the sinus floor elevation procedure.

Material and methods

The study population was comprised of patients with a loss of height in the posterior maxilla that required application of a sinus lift technique to allow rehabilitation with a fixed implant-supported prosthesis. The exclusion criteria were the presence of uncontrolled systemic disease (e.g., diabetes or blood/immune disorders) and a previous history of chronic sinusitis or allergies with a respiratory component. Seventy patients were selected for the study, 48 males and 22 females, who all signed their informed consent according to the Helsinki protocols [World Medical Association Declaration of Helsinki 2000]. The study protocol was approved by the Human Subject Review Committee at University of Granada.

Smokers were not excluded from the study but were informed that tobacco use is contraindicated in an intraoral surgery setting as it compromises the quality of the sinus lift and reduces the success rate of implants. Out of the 24 smokers enrolled in the study, 20 had stopped the habit by 2 year after the surgery.

Surgical procedure

Patients received 875 g of amoxicillin/cla-vulanic acid (1 capsule/8 h) 1 day before the surgery and 7 days post-surgically. Three patients who were allergic to penicillin received 300 mg clindamycin (1 capsule/8 h) for the same time period instead. All patients underwent surgery under local anesthesia with 1% (1:100,000) vasoco-strictor [adrenaline].

The decision to place simultaneous implants during the sinus floor elevation or at a later date depended on whether the crest had sufficient residual bone height to ensure primary stability of the implant. The minimum amount to indicate immediate implantation was 5 mm [Zinner & Small 2004]. Based on this criterion, 82 sinuses were selected to receive implants, a total of 215:135 Astra Tech [Astra Tech, Möndal, Sweden] and 80 Microdent [Microdent Implant System, Barcelona, Spain] during
sinus lift surgery, whereas the implantation was deferred in 16 sinuses (total of 48 implants: 36 Astra Tech and 12 Microdent). Hence, a total of 263 implants were used: 171 Astra Tech implants with Bio-Blast surface and 92 Microdent implants with sandblast surface treatment.

An incision was made in the palatal aspect of the alveolar crest in the edentulous area. After elevation of a full-thickness mucoperiosteal flap, access was gained to the anterior bony wall of the sinus. The bone window was obtained using a curved cortical bone collector (Safescraper®, purchased from Meta, Reggio Emilia, Italy), removing all cortical bone up to the sinus membrane and keeping it for the subsequent graft preparation. The bone collector was used to procure cortical bone that was needed for the sinus lift.

Once the membrane was exposed, it was elevated with instruments. The sinus was never lifted more than 2 cm to avoid occluding the sinusal ostium (Ziccardi et al. 1995), and was never lifted less than 12 mm to allow placement of implants of sufficient size to guarantee adequate long-term stability of the implant-supported prosthesis.

After elevation of the sinus and protecting the membrane with a flat blunt-edged metallic instrument, the alveolar crest was perforated with a very fine bur until the antral cavity was entered. The residual bone was then measured using a periodontal probe to avoid occluding the sinusal ostium (Ziccardi et al. 1995), and was never lifted less than 12 mm to allow placement of implants of sufficient size to guarantee adequate long-term stability of the implant-supported prosthesis.

Histological preparation
A 3 mm-diameter trephine was used to gather samples from patients indicated for deferred implantation (16 patients). At the time of taking the biopsies, after a minimum of 6 months of healing, bone density was similar to natural-type D1–D2 bone, according to Misch’s classification (Misch 1988). Samples were immediately immersed in buffered 4%, pH 7.7 paraformaldehyde fixative for 5 days. They were dehydrated in alcohol baths of increasing concentrations and embedded in paraffin. Sections 5 μm wide were applied onto slides. The histological analysis was performed using the standard protocols of H–E and Masson trichromic stains, which allowed the observation of individual cells and differentiation of uncalcified osteoid (Wheater et al. 1987). Finally, the sections were covered with slip covers and examined using light microscopy (Microphot-FXA; Nikon, Tokyo, Japan).

Image processing
In order to assess the total percentages of vital bone, remaining Bio-Oss® particle and surrounding connective tissue, an image analysis process, using the software Image J®, was performed on the sections obtained previously.

Implant success rate
Implant success was assessed using the criteria set up by Albrektsson et al. (1986).

Results
Surgical technique and survival of implants
No dental injuries or tears of Schneider’s membrane were noted during the procedures. No adverse events were recorded during the healing period in any of the patients, with no signs of infection. Only two Microdent implants failed before loading due to lack of osseointegration: one from the group of delayed and the other from the group of simultaneous placement. This translates to a 99.06% overall implant success rate after 24 months of function. No statistically significant differences were found between simultaneous and delayed implant placement.

Histology
Biopsy samples, taken with a 3 mm trephine, were obtained from 16 sinuses that were grafted and delayed for implant placement. Similar results could be expected in the simultaneous placement group, with regard to radiographic parameters. However, we did not take any biopsies from this group because of the ethical problems it would pose.

Histomorphometric analysis revealed that bovine bone was incorporated into new bone formation that showed an osteoid matrix (Fig. 1). Furthermore, most of the bovine bone particles remained vir-
tually unaltered, except for some areas on the surface that were partially resorbed (Fig. 2). The majority of samples showed bovine bone crystals interposed with connective tissue. In these samples, the marrow was essentially fibrous with variable vascularity [Figs 1 and 3]. Image processing revealed $34 \pm 6.34\%$ vital bone, $49.6 \pm 6.04\%$ connective tissue and $16.4 \pm 3.23\%$ remaining Bio-Oss™ particles (Table 1). Overall, bovine bone presented high biocompatibility, but demonstrated little new bone growth or graft resorption.

**Discussion**

Sinus lift procedures have allowed implants to be placed in an atrophic maxilla with high success rates. As the first description of this approach by Boyne & James (1980), numerous modifications have been published and different graft materials have been used, all aimed at technique improvements and more predictable outcomes. The composite graft (cortical autogenous bone, bovine bone and PRP mixture) used in this study showed a 99% overall implant success rate and a 100% implant success rate after 24 months of loading. This is in agreement with Moy et al. (1993), Wallace & Froum 2003 and Velich et al. (2004). Moy et al. (1993) reported that a combination of bovine bone and autogenous bone yielded better outcomes when compared with other bone graft regimes. Froum et al. (1998) found similar implant success rates when bovine bone was used with or without autogenous bone. Velich et al. (2004) compared autogenous bone, heterografts, exogenous bone and synthetic materials used alone or in combination with growth factors or morphogenetic proteins for sinus lifting. They found no differences in outcomes among these materials, with the exception of gel-state calcium carbonate, due to the high absorption of this substance. Furthermore, there was no difference between simultaneous and delayed implant placement. This is in line with recent workshop conclusions [Wallace & Froum 2003].

Our histologic data revealed $34\%$ vital bone, $49.6\%$ connective tissue and $16.4\%$ remaining Bio-Oss™ particles. This is in agreement with several studies that showed similar or higher percentages of vital bone as ours (Wallace et al. 1996; Froum et al. 1998; Valentini et al. 2000). According to Valentini et al. (2000), the residual bovine bone particles reside in the connective tissue compartment and, when combined with newly formed vital bone, can create a graft of exceptionally high density. Furthermore, the histology of explants from the maxillary sinus does not show residual bovine HA particles in contact with the implant surface, suggesting implant–vital bone contact even though bovine HA was used for sinus floor elevation [Rosenlicht & Tarnow 1999].

The rationale to use a combination of autologous bone, PRP and bovine bone was based on a detailed study of the literature. Some authors considered autologous bone from extraoral sites such as the iliac crest or tibia to be the ideal material for sinus grafting (Chanavaz 1990; Block et al. 1998). However, there are major concerns with their use, including the need for hospitalization and general anesthesia, increased surgical time and costs, higher morbidity from the second surgical site and an increased risk of intra- and postoperative complications such as fracture (harvesting from tibia) or walking difficulty (harvesting from iliac crest) [Weikel & Habal 1977; Marx & Morales 1988]. Moreover, there have been reports of ample resorption of grafts harvested from these sites [Ermis & Poole 1992; Kingsmill et al. 1999], possibly due to the embryological origin of the bone. Finally, the large amount of bone harvested at these sites was considered unnecessary to achieve a reliable sinus lift. The type of bone harvested from intraoral sites appears to be more appropriate. On the other hand, taking bone from the mandibular symphysis and ramus, while yielding adequate cortical bone (subsequently particulated for use), is frequently associated with devitalization of anterior mandibular teeth by involvement of tooth apices, changes in the facial es-

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**Table 1. Percentages per patient and mean average of vital bone, connective tissue and remaining Bio-Oss particles**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Remaining Bio-Oss</th>
<th>Vital bone</th>
<th>Connective tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. A.</td>
<td>14.3</td>
<td>16.4 ± 3.23</td>
<td>49.6 ± 6.04</td>
</tr>
<tr>
<td>A. J.</td>
<td>19.6</td>
<td>46.6</td>
<td>43.5</td>
</tr>
<tr>
<td>J. M.</td>
<td>19.8</td>
<td>34.5</td>
<td>45.7</td>
</tr>
<tr>
<td>P. L.</td>
<td>14.5</td>
<td>35.0</td>
<td>50.5</td>
</tr>
<tr>
<td>P. U.</td>
<td>17.6</td>
<td>33.6</td>
<td>48.8</td>
</tr>
<tr>
<td>J. G.</td>
<td>14.9</td>
<td>36.1</td>
<td>49.0</td>
</tr>
<tr>
<td>J-M. J.</td>
<td>12.3</td>
<td>39.5</td>
<td>48.2</td>
</tr>
<tr>
<td>F. A.</td>
<td>17.3</td>
<td>34.4</td>
<td>48.4</td>
</tr>
<tr>
<td>L. L-G.</td>
<td>23.6</td>
<td>27.6</td>
<td>48.8</td>
</tr>
<tr>
<td>C. M.</td>
<td>15.7</td>
<td>33.7</td>
<td>50.6</td>
</tr>
<tr>
<td>A. M.</td>
<td>18.9</td>
<td>35.2</td>
<td>45.9</td>
</tr>
<tr>
<td>M. V.</td>
<td>14.2</td>
<td>29.8</td>
<td>56.0</td>
</tr>
<tr>
<td>J. C.</td>
<td>19.4</td>
<td>31.3</td>
<td>49.3</td>
</tr>
<tr>
<td>M. S.</td>
<td>17.4</td>
<td>41.2</td>
<td>41.4</td>
</tr>
<tr>
<td>I. G.</td>
<td>15.8</td>
<td>33.7</td>
<td>50.5</td>
</tr>
<tr>
<td>P. G.</td>
<td>16.7</td>
<td>34.1</td>
<td>49.2</td>
</tr>
</tbody>
</table>

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thetics of the patient, possible damage to the mental or lower dental nerves and increased risk of mandibular ramus fracture. Harvesting from the tuberosity, although simple and close to the surgical field, yields an inadequate amount of highly medullary and spongy bone.

Anorganic bovine bone provides a scaffold for de novo bone formation and the slow resorption of crystals, as demonstrated in our study, helps to maintain cells carried by the autologous bone, promoting the formation of new bone within the matrix [Carmagnola et al. 2003]. Moreover, because it is deproteinized, biological risks are avoided. This bovine bone was also found to be more effective than HA as a bone substitute [Piattei et al. 1999], and it appeared to favor a more physiological remodeling toward native bone [Berglundh & Lindhe 1997]. In addition, this anorganic bovine bone has demonstrated good bio-compatibility that elicits no foreign body reaction [Denissen et al. 1980; Hislop et al. 1993; McAllister et al. 1998]. Hence, it has been widely used and associated with high clinical success rates [Carmagnola et al. 2000, 2002].

The effect of PRP in the composite graft remains to be determined. However, it is our experience that PRP enhanced the graft-handling capacity via its fibrin capacity, thus making it easier for placement of the bone graft into the sinus chamber. Other effects of PRP reported in the literature were not easily confirmed in our study as our intent was not study the effect of PRP but rather to evaluate the overall effect of the composite graft. Nonetheless, Marx et al. reported a 1.62–2.16-fold greater bone maturation of grafts mixed with PRP and a higher bone density (74.0 ± 11% vs. 55.1 ± 1%) at sites where PRP was added in comparison with grafts and sites, respectively, without PRP addition. Others have also reported positive outcomes when PRP was mixed with bone substitute, whether autogenous, allogenic or alloplastic, for sinus floor elevation [Kassolis et al. 2000; Rosenberg & Torosian 2000; Lozada et al. 2001; Fürst et al. 2003; Maiorana et al. 2003; Rodriguez et al. 2003]. On the other hand, other authors did not find PRP to be a potent bone-regenerating agent [Daneshe-Meyer et al. 2001; Jakse et al. 2003; Wiltfang et al. 2003; Butterfield et al. 2005]. Fromou et al. [2002] drew the clear conclusion that, in sinus lift techniques, there were no significant differences in the production of vital bone or in the amount of implant–bone contact interface between sinuses filled with PRP and those filled only with bovine bone [Bio-Oss®]. Future studies in this area are certainly needed.

In all cases, an absorbable collagen membrane was placed on the vestibular sinus wall to prevent invasion of the graft by soft tissues, which would reduce the amount and quality of the de novo formed mineralized tissue, as reported by various authors [Tawil & Mawla 2001; Carmagnola et al. 2003]. An absorbable membrane was selected to obviate the need for a second surgery in patients who received their implants during the sinus lift surgery, as it has been demonstrated that regeneration is equally effective whether absorbable or non-resorbable barriers are used [Avera et al. 1997].

Within the limits of this study, a composite graft that utilizes cortical autogenous bone, bovine bone and PRP mixture is indicated for use as a successful bone graft regime for sinus augmentation.

Acknowledgements: Disclaimers: The authors do not have any financial interests, either directly or indirectly, in the products listed in the study.

References


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