Maxillary Sinus Floor Elevation Using Platelet-Rich Plasma Combined With Either Biphasic Calcium Phosphate or Deproteinized Bovine Bone

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Purpose: Maxillary sinus floor elevation procedure has the objective of augmenting available bone in atrophic posterior maxilla to allow dental implants placement. The main aim of this prospective study was to evaluate clinically and histomorphometrically the performance of biphasic calcium phosphate (BCP) used in conjunction with platelet-rich plasma (PRP) compared with demineralized bovine bone matrix (DBBM) and PRP in sinus floor elevation surgery.

Materials and Methods: Patients candidate to sinus floor elevation were treated using either BCP and PRP or DBBM and PRP. Biopsies were retrieved using trephine bur during implant placement surgery 6 months after grafting. Clinical success of implants was evaluated 1 year after prosthetic delivery. Histomorphometric analysis was performed assessing the relative volume of newly formed bone.

Results: A total of 20 patients were recruited, and 20 sinus augmentation procedures were performed, 10 for each group. A total of 42 implants were placed. One year after prosthetic loading a 100% implant survival rate was reported in both groups. Histomorphometric analysis revealed that the mean amount of new bone formation was 18.6 ± 3.3% in BCP group and it was 21.9 ± 4.9% in DBBM group, without statistically significant difference. In BCP group a greater amount of collagen type I was found with respect to DBBM group.

Conclusions: Both grafting materials led to an excellent performance regarding implant survival rate after 1 year follow-up, without any significant adverse sequelae. A similar capability of inducing new bone formation was observed in both groups, even though the higher quantity of collagen type I in BCP group may suggest a greater potential for bone formation over time as compared with DBBM.

Key Words: Biphasic calcium phosphate, bone graft, histomorphometry, maxillary sinus augmentation

Poster ior maxilla bone atrophy, following teeth extractions, could seriously limit the possibility of placing dental implants due to the insufficient amount of available bone volume. Moreover, the maxillary sinuses could be extensively pneumatized in elderly edentulous subjects, further reducing the bone available to place dental implants.1

Bone augmentation procedures were proposed to remedy the insufficient bone volume in posterior maxilla.2 Vertical bone augmentation aims at addressing the vertical discrepancy in bone volume, which follows the bone resorption process. The use of both inlay and onlay grafts was described in scientific literature, also in combination with Le Fort I surgery.3,4 Vertical bone augmentation with the use of particulate bone grafts and barrier membranes was also successfully used.3

The augmentation of available bone can be obtained also through the cranial dislocation of the maxillary sinus floor, allowing bone formation in the so-created space.

Maxillary sinus lifting can be achieved, when more than 5 mm of residual bone height is available, through a crestal approach that was demonstrated to be successful in terms of implant survival rate6–8 also in the medium and long terms.9

In patients of less than 5 mm of residual bone height a lateral approach is indicated.10 This technique was first proposed in a series of lectures by Tatum in the 1970s11 and subsequently by Boyne in 1980.12 Several systematic reviews of the literature have demonstrated that implants placed in augmented sinus may achieve a survival rate of more than 90% even in long-term investigations.10,13,14

A number of biomaterials were used and studied for lateral approach maxillary sinus floor elevation (LASFE) surgeries. Autogenous bone,3,15 synthetic bone substitutes,16 allografts,17 and xenografts18,19 were widely used alone or in combination with the aim of filling the space created by the floor elevation, stabilizing the blood clot, and allowing new bone formation.

Among all synthetic biomaterials, biphasic calcium phosphate was widely used and described in scientific literature. Biphasic calcium phosphate contains (in variable proportions) both synthetic hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) to
improve its features as bone substitute material. In fact, it was demonstrated that HA can have longer resorption time than β-TCP, serving for bone volume maintenance over time. On the other side, the relatively faster resorption time of β-TCP\(^{16}\) might help the colonization of the scaffold by osteoprogenitor cells.

Recently, a number of clinical studies and systematic reviews investigated the use of platelet derivative as an adjunct to bone substitute material in LASFE surgery.\(^{21-23}\) Platelet-rich plasma (PRP) contains a high concentration of platelets that, after activation, release a large number of molecules that are involved in the processes leading to hard and soft tissue healing.\(^{24}\)

The aim of this prospective study was to compare a biphasic calcium phosphate (BCP) material (composed by 40% HA and 60% β-TCP) contained in collagen scaffold (10%) versus deproteinized bovine bone mineral (DBBM), both used in combination with platelet concentrate in LASFE surgery, evaluating implant survival rate. Also, histological and histomorphometric analysis of 2 patients per group were performed to evaluate the characteristics of the regenerated bone.

**METHODS**

The patients enrolled in this study were treated following the principles described in the Helsinki Declaration and further modifications.\(^{25}\) The study protocol was approved by the Review Board of the IRCCS Istituto Ortopedico Galeazzi, in Milan, Italy (n: L.2025). This paper was written following the CONSORT guidelines for reporting clinical trials.\(^{26}\)

The controlled clinical trial reported in this paper has a ratio 1:1. The inclusion criteria for patients were

- more than 18-year old and able to understand and sign an informed consent
- absence of any systemic pathologies or conditions that were a relative or absolute contraindication to LASFE surgery (subjects had to be classified as ASA-1 or ASA-2 following the classification proposed by the American Society of Anesthesiology)
- presence of a posterior maxilla edentulism with less than 4 mm of residual bone height in the edentulous area (class C or D following the Lekholm & Zarb classification\(^{27}\))
- nonsmokers or smoking less than 5 cigarettes a day

The exclusion criteria were

- pregnancy or nursing status
- occurrence of any surgical complications that can cause the abortion of intervention or any other surgical procedure than standard LASFE (ie, perforation of Schneiderian membrane that required the use of barrier membranes for management of the perforation)
- presence of any pathosis of maxillary sinus (eg, sinusitis) as evaluated through presurgical assessment

**Surgical Procedure**

Surgeries were all performed by the same operator (S.T.) that has more than 10 years of experience in oral surgery. All procedures were delivered in the dental clinic of the IRCCS Istituto Ortopedico Galeazzi, Milan, Italy.

Before intervention all surgeries were planned through clinical and radiological investigation performed with the aid of cone beam computed tomography scan.

After induction of local anaesthesia with Articaine 4% + epinephrine 1:100,000, a mucoperiosteal flap was elevated approximately from the region of first premolars to the region of second molars. One or 2 vertical release incisions were applied to obtain adequate flap mobility. After elevation, the extension of the window to access maxillary sinus was obtained referring to cone beam computed tomography scans. Using a round bur, abraision of the sinus wall was obtained until surgical access was achieved. The sinus membrane was elevated following the protocol described by Taschieri et al.\(^{28}\) Briefly, at first pure platelet-rich plasma without leukocytes (P-PRP) was prepared in accordance with the protocol recommended by the manufacturer of the centrifuge (PRGF System, BTI Biotechnology Institute, Vitoria, Alava, Spain) and described in previous reports.\(^{28}\)

The membrane detachment was performed beginning from the mesial wall and the procedure was continued until complete detachment of the membrane from the sinus walls, obtaining an adequate space for the bone substitute placement. The absence of membrane perforation or injury was assessed by the evaluation of the respiratory sinus function (Valsalva manoeuvre).

In the control group a combination of DBBM (BioOss, Geistlich Pharma, Wolhusen, Switzerland) and liquid PRP was gently positioned in the space below the lifted membrane, taking care to avoid damaging the sinus membrane.

In the test group the bone substitute material was composed by a homogenous mixture of 60% HA and 40% β-TCP contained in a freeze-dried collagen scaffold (Type I and III collagen) (MATRI BONE, Biom’Up, Saint-Priest, France) embedded with liquid PRP. After filling the cavity, the flap was repositioned and sutured.

All the patients were instructed to avoid any action that would suddenly increase or lower the air pressure in the maxillary sinus for at least 10 days after surgery.

Postsurgical instructions were also provided to limit the occurrence of complications in the healing phase. Moreover, the patients were suggested to gentle rinse twice a day with 0.2% chlorhexidine digluconate solution for 10 days. Nonsteroidal analgesics and antibiotic therapy (Amoxicillin 1 g twice a day for 6 days) were prescribed to all patients.

Sutures were removed after 10 days. Then patients were scheduled for second-stage surgery (implant placement) 6 months after LASFE intervention. In 2 patients per group, while preparing the sites for implant positioning a trephine bur (outer diameter 4 mm, inner diameter 3.4 mm) was used, obtaining bone cores for histological and histomorphometric analysis.

Oral hygiene instructions were provided at each follow-up visit and professional oral hygiene was administered 6 months and 12 months after the placement of the prosthesis.

**Histologic and Histomorphometric Analysis**

Biopsy samples were removed and fixed in 10% (v/v) phosphate-buffered formaldehyde for 48 hours, then dehydrated in a graded series of 50% (v/v), 70% (v/v), 95% (v/v), and 100% (v/v) ethanol series, then immersed in a 4% paraformaldehyde solution and ethanol (1:1). After dehydration, all samples were dried, dehydrated, and embedded in paraffin and cut into 4-μm-thick sections. These sections were then rehydrated in a series of 70% (v/v), 50% (v/v), 30% (v/v), and 100% (v/v) ethanol series, and finally dehydrated in a series of ethanol and acetone. Sections were then mounted onto slides, deparaffinized with xylene, and stained with hematoxylin and eosin. The images were analyzed using the ImageJ software (version 1.41, National Institutes of Health, Bethesda, MD). For the determination of the vital bone content, a×25 magnification was used, evaluating the complete section for each patient; approximately 7 mm\(^2\) were examined. The newly formed bone, residual of bone substitute particles (DBBA and BCP), and soft tissue areas were measured semiautomatically and expressed as percentage of the total area.
Immunohistochemical Analyses

Other 4-μm-thick sections were used for immunohistochemical analyses. After rehydration, heat-induced antigen retrieval was performed by treating the sections in citrate buffer, pH 6.0, in a microwave oven. Sections were then washed 3 times in phosphate buffered saline (PBS, pH 7.4). To block endogenous peroxidase activity, sections were incubated in an aqueous solution of 1% H2O2 for 30 minutes at room temperature and afterward washed 3 times in PBS. Sections were then incubated overnight with either mouse anticollagen type I antibody (Chondrex Inc, Redmond, WA; 1:500) or osteopontin (Santa Cruz Biotechnology, Dallas, TX; 1:250). Antigen–antibody complexes were detected with a peroxidase-conjugated polymer that carries secondary antibody molecules directed against mouse immunoglobulins (EnVision+; Dako, Carpinteria, CA) applied for 90 minutes at room temperature. Appropriate washing with PBS was performed between each step and all incubations were carried out in a moist chamber. Peroxidase activity was detected with diaminobenzidine (DAB, Dako) as the substrate.

For both the immunohistochemical procedures the sections were weakly counterstained with Mayer hematoxylin, dehydrated, and permanently mounted. The specificity tests for the collagen type I or osteopontin antisera were verified by incubating sections with PBS instead of the specific primary antibody; PBS instead of the secondary antibodies. The results of these controls were negative (ie, staining was abolished). Photomicrographs were taken with an Olympus BX51 microscope (Olympus) equipped with a digital camera and final magnifications were calculated.

Outcomes

The primary outcomes evaluated in this study were
- implant survival rate 1 year after prosthetic loading. For assessing survival, implant had to be stable, without showing acute infection and successfully supporting a functional prosthesis.
- occurrence of surgical complications as acute sinusitis or any infective process affecting the surgical site in the postoperative period

The secondary outcomes evaluated through the histological and histomorphometric analysis were
- percentage of newly formed bone in the sample;
- presence of type-I collagen and osteopontin in the sample.

Further outcomes were the global satisfaction of the practitioner and of the patient, as assessed through a questionnaire based on a 0 to 10 scale, where 0 represented the most unsatisfactory option and 10 the most satisfactory one. The questionnaire was administered immediately after the surgical procedure.

Patients were scheduled for follow-up visits at 6 and 12 months after the placement of the definitive prosthesis. In each follow-up visit the evidence of implant failures was evaluated as well as the occurrence of any biological or surgical complications. Periapical radiographs were taken at the 12-month follow-up visit and in the presence of sign and symptoms of perimplant tissues inflammation (p.e. bleeding, swelling, pain).

Statistical Methods

Statistical analysis was performed by a blinded operator (SC) using a software package (R 3.0.2, Institute for Statistics and Mathematics Wirtschaftsuniversitat Wien, Wien, Austria). The implant survival rate and occurrence of complication were presented narratively as a percentage value. Quantitative variables presented as mean values ± standard deviation.

RESULTS

A total of 20 patients accounting for 20 LASFE procedures met the inclusion criteria and were included in the present study. The age of subjects ranged from 49 to 69 years. Twelve subjects were women and 8 were men and none of them was smoker. Ten subjects were included to control group and 10 to test 1. No randomization was made.

A summary of demographic data of subjects included is presented in Table 1.

A total of 42 implants (39 placed in the graft) were placed. Implant distribution is shown in Table 2. After 1 year from prosthetic loading none of the implants failed, obtaining a 100% implant survival rate in both groups. None of the implants and of the patients experienced any biologic complication.

In the DBBM group the mean practitioner satisfaction was 8.6 ± 0.5 and the mean patient satisfaction was 9.4 ± 0.5, whereas in the BCP group it was 9.0 ± 0.0 for practitioners and 9.6 ± 0.5 for patients without any significant difference.

Histologic and Histomorphometric Analysis

Newly formed bone was in close contact with both the 2 tested biomaterials with no gaps at the interface bone-biomaterial, in many fields of the slides (Fig. 1A and B). The newly formed bone was strongly stained with Masson trichrome and showed a trabecular appearance and no inflammatory infiltrate was evident at higher magnification (Fig. 1C and D). Moreover, the biomaterial particles revealed to be present. Most of the residual biomaterial was present in the apical portion (Fig. 2). Histomorphometric analysis revealed that the mean amount of new bone formation was 18.6 ± 3.3% in collagenated biphasic calcium phosphate group and it was 21.9 ± 4.9% in DBBM group, without statistically significant difference.

Immunohistochemical Analyses

Various amounts of newly formed bone were mainly observed in both the experimental groups, as revealed by immunohistochemistry for both collagen type 1 and osteopontin expression. Immunopositivity of new bone tissue for these osteogenic proteins was observed in conjunction with the 2 different biomaterials used in the study (Fig. 2). In particular, a slighter immunoreactivity in tissue matrix

### Table 1. Demographic Data of Treated Patients

<table>
<thead>
<tr>
<th></th>
<th>BCP</th>
<th>DBBM</th>
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<tr>
<td>Age (yr)</td>
<td>48.2</td>
<td>46.7</td>
</tr>
<tr>
<td>Men/women</td>
<td>6/4</td>
<td>6/4</td>
</tr>
<tr>
<td>BCP, biphasic calcium phosphate; DBBM, demineralized bovine bone matrix.</td>
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### Table 2. Implant Distribution

<table>
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<tr>
<th></th>
<th>17</th>
<th>16</th>
<th>15</th>
<th>14</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
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</thead>
<tbody>
<tr>
<td>BCP</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>DBBM</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOT</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>10</td>
<td>4</td>
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BCP, biphasic calcium phosphate; DBBM, demineralized bovine bone matrix.
The present investigation showed that similar clinical results were achieved after 1 year from prosthetic loading as regard implants placed in maxillary sites after LASFE surgery with 2 different biomaterials. The absence of biological complications is a promising outcome, even though the follow-up period is short. The histological and histomorphometric analysis showed that, even though a difference could be observed in the percentual amount of new bone in favor of the specimens from the control group, the findings about the greater presence of type I collagen and osteopontin in the test samples could suggest a certain amount of tissue remodeling that can be hypothesized to lead to further bone formation over time.

Mature osteoblasts as well as preosteoblasts are able to synthesize specific bone extracellular proteins by a specific gene activation over time. The first bone protein synthesized during the active proliferation phase is collagen type I. Subsequently, in the postproliferative period, osteoblasts secrete several proteins that are associated to in vivo mineralization. One of these extracellular bone proteins is osteopontin that is expressed 25% during active proliferation, but mostly during early mineralization, and is present in large amounts of immature bone. For these reasons, from the present histological findings we can hypothesize a different timing of mineralization using different bone substitutes. The latter of course needs to be confirmed by histological investigation in a larger sample size. Immunohistochemical evaluation was rarely performed in previous investigations assessing the performance of BCP in the LASFE procedure. The estimation of the osteopontin and collagen type I amount performed in the present study represents a novel insight into the understanding of the regenerative potential of BCP material.

Some limitations could be highlighted in this pilot study. One is the relatively small sample size. In fact, a biopsy could not be performed in all treated patients due to ethical reasons and respect of the patients’ willingness. The reduced number of patients undergoing histological analysis represents a limit to the external validity of the findings. Furthermore, the follow-up period is rather short, also preventing a direct generalization of the results.

Several studies reported clinical outcomes after the LASFE procedure using a combination of HA and TCP. The results reported in those studies confirmed that biphasic calcium phosphate biomaterial could be safely used for the LASFE procedure leading to clinical results that can be considered comparable to those reported in the present investigation. Lindgren et al. published in 2012 the results of a randomized clinical trial comparing the use of biphasic calcium phosphate biomaterial and DBBM in LASFE procedures. After a follow-up period of 3 years only 1 implant per group was considered failed, resulting in an overall implant survival rate of 96.8%.

Another study with a short follow-up period (6 months of graft healing plus 3 months from implant placement) in 6 patients reported osseointegration for all implants placed in sites grafted with 100% biphasic calcium phosphate consisting of a mixture of 60% HA and 40% beta-TCP. Moreover, the authors reported an average 27.3 ± 4.9% (standard deviation) of bone volume/total volume evidenced from histomorphometric analysis, and radiologic evidence of new bone formation after a 6-month healing period. Clinical and histomorphometric evaluation of a macroporous BCP composed of a combination of HA and TCP was reported also by Mangano et al. They used a HA:TCP 60:40 proportion for 12 LASFE surgeries in 10 patients and found a newly formed bone of 28.3% ± 2.7% after a 6-month healing period. In addition, all 30 implants placed were clinically in function 1 year after implantation. The same authors in another study evaluated radiologically, histologically, and histomorphometrically a combination of HA and TCP in the ratio 30:70 used in LASFE procedures in 12 patients. After 6 months of healing they found 29 ± 3% of newly formed bone and 26 ± 2% of residual graft material.
No implant failures or clinical complications after using a graft composed by HA and TCP (in a 60:40 ratio) in the LASFE procedure in 12 patients were reported also by Kollerman et al.39 They found a 26.4% newly formed bone after 9 months of healing. The histological analysis of the test group in the present investigation reported results that were comparable to those presented in scientific literature.32–38 However, the mean percentage of newly formed bone in grafts composed by a combination of HA and TCP reported in the literature is extremely variable, ranging from 20.6% after 6 months of healing in 64 patients29 to 37.5% after 8 months in 6 patients.38 The percentage of the residual biomaterial is also heterogeneous ranging from 15.8% after 5 months in 14 patients15 to 33.7% after 9 months in 15 patients.38 In the present study, the histomorphometric results between the test and the control group were very similar, though no statistical comparison was feasible due to the low number of samples analyzed. Anyway also another published comparative study confirmed such an observation.31

The beneficial use of PRP as an adjunct to bone substitute material for LASFE procedures is controversial.22 In fact, some studies reported a positive effect in enhancing both soft and hard tissue healing,23,34,36 whereas others could not confirm such apparent benefit.22 In the present study, PRP was used in both groups with the aim of reducing the impact of the surgeries on postoperative quality of life, as it was proved in a previously published study about patients treated with the same technique.21 As regard the evaluation of the presence of Type I collagen, it is known that such protein synthesis could be enhanced by a number of growth factors such as TGF-b, IGF, and FGF and cytokines such as TNF-a and IL-1.22 Thus, it can be hypothesized that the BCP was deposited in the grafts more homogeneously, even though this should be proved by a larger number of histological analyses.

In conclusion, within the framework of this pilot study, the use of biphasic calcium phosphate led to clinical and histomorphometric results that were comparable to those of DBBM when used for LASFE surgery in combination with PRP. The emerging evidence about some markers of bone growth (such as osteopontin and type I collagen) should be further explored in studies with wider sample size and longer follow-up to better understand their predictive value.

COMPLIANCE WITH ETHICAL STANDARDS

All the procedures performed in the study were in accordance with the ethical standards of the research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

REFERENCES
