The aim of this histomorphometric study was to compare the outcome of sinus floor augmentation procedures using bovine bone mineral and a xenograft enriched with gelatin and a polymer. In 20 patients a single sinus floor elevation procedure with a lateral window approach was performed. In half of the patients, sinuses were grafted with a deproteinized bovine bone mineral—DBBM (control group)—while in the remaining 10, a xenograft enriched by polymer and gelatin—NBS (test group)—was applied. In the DBBM group, histomorphometric analysis revealed 23.14 ± 10.62% of lamellar bone, 19.43% ± 9.18% of woven bone, 23.35% ± 6.04% of osteoid, 17.16% ± 6.13% of biomaterial particles, and 16.93% ± 9.78% of medullary spaces. In the NBS group, histomorphometric analysis found 39.64% ± 12.02% of lamellar bone, 16.28% ± 7.75% of woven bone, 17.51% ± 4.87% of osteoid, 12.72% ± 5.36% of biomaterial particles, and 13.84% ± 6.53% of medullary spaces. Differences between groups for proportion of lamellar bone (P = .004) and osteoid (P = .0287) were statistically significant. Inflammatory infiltration was appreciated only in the NBS group. The enriched xenograft showed a statistically significant higher proportion of lamellar bone and osteoid; however, this was accompanied by an accentuated inflammatory infiltrate. Int J Periodontics Restorative Dent 2021;41:579–586. doi: 10.11607/prd.4630

Following tooth extraction, maxillary bone atrophy due to loss of function is usually seen together with micro-trauma during extraction.1 In the posterior maxilla, sinus pneumatization is also common.2

Implant-supported rehabilitation is a predictable treatment alternative to restore missing teeth. However, implant insertion requires adequate bone volume.3 Short dental implants may be considered a viable treatment option for cases with at least 4 to 5 mm of residual bone.4 Yet, in many cases, the available bone height under the sinus floor does not allow for implant placement, requiring bone augmentation procedures to overcome this limitation.5-7

Maxillary sinus floor elevation (MSFE) is intended to augment the bone volume in the latero-posterior maxillary areas.8,9 Depending on the amount of residual bone and the desired outcome, either a transcrestal or a lateral opened approach may be applied.10,11 Several systematic reviews in the literature have reported favorable outcomes in bone volume gain12,13 and implant survival rates11,14,15 after MSFE procedures, both in short- and long-term evaluations.

Among the grafting biomaterials in MSFE procedures, autogenous bone has been considered the gold standard for years.16 Nonetheless, bone substitutes have been shown...
to be as effective as autogenous bone, even in extremely atrophic cases. Moreover, different materials could have different resorption times, thus optimizing the characteristics of the bone graft to the needs of the particular clinical case. Maxillary sinus floor augmentation by a lateral approach together with implant placement without applying any biomaterial is feasible, as blood clot stability could be sufficient to preserve a secluded space for obtaining bone regeneration. However, there are several anatomical situations in which this approach cannot be applied.

Two systematic reviews evaluated the histomorphometric results of new bone formation in MSFE procedures with different biomaterials, reporting no clear advantage of any graft. More recently, new bone substitutes were proposed with a hybrid formulation, adding polymers or other substances to xenografts in order to enhance the bone augmentation procedures. Such materials were hypothesized to enhance new bone formation by some authors reporting data on a small sample of subjects.

The aim of the present comparative prospective study was to evaluate whether one new bone substitute with adjunctive polymers could lead to higher new bone formation as compared to deproteinized bovine bone mineral (DBBM) when used in MSFE.

Materials and Methods

The study protocol was approved by the Scientific Board of the IRCCS Istituto Ortopedico Galeazzi in Milan, Italy, in 2018 and was included in one research project approved by the Italian Ministry of Health. All study phases were performed in accordance with the Declaration of Helsinki, and all patients signed an informed consent form before beginning the study.

This prospective, nonrandomized clinical study comprised 20 patients in which 20 MSFE procedures were performed (one procedure per patient).

Inclusion Criteria

Inclusion criteria were as follows: (1) American Association of Anesthesiologists classification of ASA I or ASA II; (2) single or multiple edentulous in the latero-posterior area of the maxilla (premolars/molars); (3) < 5 mm of residual bone height; (4) no previous regenerative procedures in the site of intervention; (5) nonsmokers, former smokers, and those smoking fewer than five cigarettes a day; and (6) no sinus pathology that could affect treatment outcome.

Allocation

Ten sinuses (control group) were grafted with DBBM while the other 10 (test group) were grafted with a xenograft enriched by polymer and gelatin (NBS).

Interventions

The same experienced surgeon (S.T.) performed all surgical interventions. The DBBM (Bio-Oss, Geistlich) grain size was 0.25 to 1 mm. The NBS graft material was composed of bovine bone matrix and a polymer (L-lactic-co-e-caprolactone) in a gelatin scaffold with a grain size of 0.25 to 1 mm (SmartBone, Industrie Biomediche Insubri). Deciding the graft type for each patient was established by alternate allocating of subjects to the control group or the test group. All patients followed preoperative diagnostic CBCT scanning. The surgical intervention was performed following a standard procedure.

Briefly, after local anesthesia (with articaine 4% and adrenaline 1:100,000), a trapezoidal flap was elevated to access the sinus, which was identified by careful preoperative evaluation of the CBCT scans. Access was created by eroding the lateral bone wall with a round diamond bur mounted in a low-speed handpiece connected to physiodispenser (American Eagle Instruments), taking care to avoid injuring the sinus membrane. Detachment of the membrane was carefully performed, and the created cavity was filled with the biomaterial. After finalizing the sinus grafting, the access window was covered with a resorbable collagen membrane (GRAFT collagen membrane, Alpha-Bio Tec). The flap was repositioned and sutured with interrupted sutures (Ethilon, Ethicon, Johnson & Johnson). Patients were instructed to avoid any activity that would abruptly raise or lower pressure in the sinus cavity for 3 weeks after surgery, such as sneezing with their mouth closed, blowing their
nose, traveling on an airplane, sucking through a straw, diving, blowing balloons, or playing a wind instrument. Furthermore, patients were instructed to avoid vigorous mouth rinsing, hard and hot foods, strenuous exertion, smoking, and touching the gums for at least 3 days after surgery. Patients were instructed to gently rinse with a 0.2% chlorhexidine digluconate solution twice a day for 10 days. All patients were prescribed nonsteroidal analgesics to be self-administered as needed. Antibiotic therapy with amoxicillin (1 g, twice a day for 6 days) was prescribed to all patients.

Six months after the MSFE, radiographic and clinical evaluations were performed, and implants were placed in the previously augmented areas. At this time, bone core samples were retrieved with a 3.2-mm-diameter trephine bur in the direction of implant site preparation.

Histologic Analysis

Retrieved tissue samples were fixed in 40% ethanol solution for 48 hours at 4°C and then dehydrated with ethanol, propanol, and xylene for 48 hours. After dehydration, samples were infiltrated with a mixture of ethanol and acrylic resin in decreasing ratios (3 alcohol:1 resin, 1:1, 1:3, pure resin), and finally embedded in pure methyl-methacrylate resin (Technovit 7200 VLC, Kulzer). Using a mounted diamond blade, the vials containing the embedded samples were cut longitudinally in a coronal-apical direction and grinded to achieve 70-µm thickness. Two sections from each biopsy sample were mounted and then stained with toluidine blue on a hot plate. After drying, each slide was analyzed under light microscope at different magnifications (×2, ×4, ×10, ×20, and ×40). The highly mineralized matrices showed a brown color (biomaterial with high degree of mineralization), while the less-mineralized matrix showed a purple/dark blue color (osteoid, woven bone, and polymer); in all sections, the connective tissue was stained light blue. Each sample was digitally recorded for histomorphometric analysis (tissue fractions).25 The histomorphometry was performed on the overall image of the most representative section of each biopsy sample. ImageJ software (National Institutes of Health) was used for outcome measures.

Outcome Measures

The primary outcome was the new bone volume proportion (NBV). The secondary outcomes were: (1) bone substitute volume (BSV); (2) proportion of connective tissue volume (CTV) in the specimen; (3) occurrence of complications; and (4) alveolar bone height reduction (ABHR) available at the moment of implant placement, measured by comparing periapical radiographs immediately after MSFE and before implant placement.

Statistical Analysis

Descriptive statistics provided mean and SDs for continuous variables. Shapiro-Wilk test was applied to test normality of distributions of primary (NBV) and secondary (BSV and CTV) outcome variables. Differences between groups for normally distributed variables were tested by means of Student t test. Statistical significance was established at $P < .05$.

Results

Demographic data are presented in Table 1. No surgical complications occurred. Results of the histomorphometric analysis are presented in Table 2.

The qualitative evaluation of the sections included graft integration, the presence of osteoclast cells, and coating polymer degradation (Figs 1 and 2). In both groups, the grafted bone substitute appeared surrounded by newly formed bone, in close contact to the biomaterial, and was slightly mineralized (Figs 3 and 4).

In the DBBM group, multinucleated cells (probably osteoclasts) were clearly identified in close contact at the interface and between the new bone and the DBBM particles (Fig 5). In the NBS group, macrophages were detected both at the interface between bovine bone granules and newly formed bone but also in close contact with the graft (Fig 6).

In two samples in the NBS group, the polymer-coated material appeared to be surrounded by a dense layer, rich in cells (inflammatory cells). In both groups, good vascularization of the newly formed tissues could be observed (Fig 7).
Table 1 Patient Demographics

<table>
<thead>
<tr>
<th></th>
<th>DBBM group (n = 10)</th>
<th>NBS group (n = 10)</th>
<th>Total (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (M/F)</td>
<td>6/4</td>
<td>6/4</td>
<td>12/8</td>
</tr>
<tr>
<td>Age, y</td>
<td>60.2 ± 7.8</td>
<td>57.9 ± 11.2</td>
<td>59.1 ± 9.4</td>
</tr>
<tr>
<td>Baseline RBH, mm</td>
<td>2.6 ± 0.9</td>
<td>2.6 ± 1.1</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Edentulism, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Partial</td>
<td>6</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Full</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

DBBM = deproteinized bovine bone mineral; NBS = xenograft enriched by polymer and gelatin; M = male; F = female; RBH = residual bone height.

Table 2 Histomorphometric Analysis Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Lamellar bone</th>
<th>Woven bone</th>
<th>Osteoids</th>
<th>Graft particles</th>
<th>Medullary spaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBBM (n = 10)</td>
<td>23.14% ± 10.62%*</td>
<td>19.43% ± 9.18%</td>
<td>23.35% ± 6.04%**</td>
<td>17.16% ± 6.13%</td>
<td>16.93% ± 9.78%</td>
</tr>
<tr>
<td>NBS (n = 10)</td>
<td>39.64% ± 12.02%*</td>
<td>16.28% ± 7.75%</td>
<td>17.51% ± 4.87%**</td>
<td>12.72% ± 5.36%</td>
<td>13.84% ± 6.53%</td>
</tr>
</tbody>
</table>

DBBM = deproteinized bovine bone mineral; NBS = xenograft enriched by polymer and gelatin. Values are shown as mean percentages of all analyzed samples within a study group.

*Statistically significant (P = .004).
**Statistically significant (P = .0287).

Fig 1 DBBM group (toluidine blue staining; ×20 magnification). Green arrows identify newly deposited lamellar bone in the mineralization phase, surrounding blocks of biomaterial.

Fig 2 NBS group (toluidine blue staining; ×20 magnification). Red circles indicate the graft particles, while green arrows identify newly deposited lamellar bone. Orange circles indicate the presence of polymer material.
In the DBBM group, the inflammatory infiltrate was only a sporadic event where few inflammatory cells of small size were detected, predominantly lymphocytes and granulocytes. By contrast, infiltrates in the NBS group were observed in 2 of the 10 specimens.

Remodeling the graft after MSFE caused an ABHR of 2.7 ± 0.9 mm in the NBS group and 1.2 ± 0.5 mm in the DBBM group, which was statistically significant ($P < .05$).

**Discussion**

The present study revealed that the percentage of lamellar, mature bone was higher in the test group. The main limitation of the study was its design, specifically the non-randomized allocation and sample...
size. However, demographic characteristics of both groups were similar at baseline, both without any systemic disease or condition that might have affected the outcomes. With regard to sample size, even though a sample size calculation was not performed before the study began, the calculated power of the included sample (after collecting data) was 0.83, thus justifying the sample size. Finally, heterogeneity between the distribution of restoration type between groups was found. It was assumed that, despite such difference, the influence on histomorphometric outcomes could be negligible.

The aim of the study was to evaluate the histologic and histomorphometric outcomes, not the clinical and radiographic extent of bone augmentation. As previously mentioned, no complications were recorded, and all implants were placed as planned.

A systematic review reported that, after 6 months of healing following MSFE procedures with DBBM, averages of 28.3% of mature bone and 27.8% of residual biomaterial could be observed. The results of the present study were comparable to another systematic review that evaluated histomorphometric outcomes of different biomaterials in MSFE procedures. The significant proportion of immature vital bone in close contact with the biomaterial particles and the mature bone indicated a high degree of integration.

Whilst reports concerning the use of DBBM alone or in combination with other bone substitutes are extensive, few studies have evaluated the biomaterial used in the NBS group (SmartBone) in oral and maxillofacial surgery. One study evaluated the outcomes of NBS used as grafting material in five cases with MSFE procedures. Contrary to the present findings, that study reported that a majority of the bone substitute material was not detectable 6 months after implantation. Moreover, the authors stated that the biomaterial enhanced new bone formation, but this was not observed in the present study. Additionally, the present findings cannot confirm the reported histomorphometric results of other studies with 6-month follow-ups, maybe due to the larger sample size and the different methodology adopted in the present research. Other reports concerning the use of SmartBone biomaterial dealt with customized grafts in maxillofacial surgery for calvarial and zygomatic reconstructions. Although good outcomes were reported in those case reports, they cannot be generalized.

The present study found differences in the amount of lamellar bone and osteoids between the two groups; it can be hypothesized that such results are related to the different osteogenic properties between NBS and DBBM. Because the osteoconductive properties of polymeric materials have not been clearly demonstrated, one can assume that this may have influenced the histomorphometric results.
Another important issue to be considered is the presence of an inflammatory infiltrate surrounding the polymer molecules, seen in two samples in the NBS group. Such findings should be further evaluated to elucidate the frequency of occurrence and whether it is part of the material's integration/resorption process or related to a foreign-body reaction to the polymer.

Conclusions

Despite the limitations of the present study and although the proportion of lamellar bone was statistically significantly higher in the NBS group than the DBBM group, it is still not clear which bone substitute performed better in the overall evaluation. The histomorphometric analysis showed that integration of the biomaterials was not complete 6 months after intervention and that residual polymer was found in the samples, sporadically associated with an inflammatory infiltrate.

Acknowledgments

The authors declare no conflicts of interest.

References


