Alveolar Ridge Preservation with Deproteinized Bovine Bone Mineral and Xenogeneic Collagen Matrix: A 12-Month Clinical and Histomorphometric Case Series

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The aim of the present study was to evaluate the efficacy of alveolar ridge preservation (ARP) protocol using deproteinized bovine bone mineral (DBBM) covered with a collagen matrix (CM), as well as to clinically and histologically analyze the alveolar bone healing at 12 months, prior to implant placement. Six patients had bone biopsy samples harvested and underwent implant placement at 12 months following ARP. At 12 months, DBBM granules represented a mean 29.52% ± 6.09% of the specimens and were embedded in the newly formed bone, which represented a mean 27.72% ± 5.64% of the sample. Data suggest that 12 months of ARP using DBBM granules covered with a CM may be considered a predictable technique providing favorable conditions for implant placement in the anterior maxilla. Int J Periodontics Restorative Dent 2021;41:423–430. doi: 10.11607/prd.4606

Tooth extraction results in a series of biologic processes that lead to soft and hard tissue healing.¹ Healing of mucosal tissues occurs in a period of 6 to 8 weeks, whereas bone tissue healing may require up to 1 year to accomplish complete tissue mineralization.² However, the process of alveolar ridge healing is associated with volumetric and morphologic changes due to bone resorption, mainly involving the socket buccal plate both in horizontal and vertical dimensions.³–⁵ Most of the ridge width reduction occurs during the first 3 months and continues for up to 6 months after tooth extraction.⁶,⁷ Several studies identified a buccal plate ≤ 1 mm thick as a critical predictive value for significant bone resorption leading to negative changes in alveolar ridge width and height.⁸,⁹ This critical thickness is present in 71% of maxillary anterior teeth and premolars.¹⁰ Furthermore, many factors affect the amount of postextraction bone resorption, independent of a flap or flapless approach.¹¹,¹²

A number of techniques have been proposed to compensate for postextraction ridge changes and to ensure that adequate ridge dimensions are preserved for implant placement.¹³–¹⁵ Several studies have been conducted evaluating alveolar ridge preservation (ARP) with different socket-filling materials,
often in combination with barrier membranes or matrices. In this regard, encouraging results have been reported with the use of xenografts, and deproteinized bovine bone mineral (DBBM) has been successfully used to preserve ridge dimensions following tooth extraction in both animal and human studies.

The efficacy of ARP using DBBM in granular form or implemented with 10% collagen (DBBM-C) has been tested in association with an autologous epithelial-connective tissue graft, collagen membrane, and collagen matrix (CM), each showing comparable clinical results. Nevertheless, the use of DBBM seems to be associated with delayed alveolar bone healing.

However, no specific protocol for ARP has been proven to be superior to others, and there still is a lack of evidence regarding the optimal timing for implant placement following ARP. In light of these findings, the present study aimed to evaluate the clinical efficacy of ARP using DBBM covered with xeno- geneic CM and to histologically and histomorphometrically analyze alveolar bone healing at 12 months, prior to dental implant placement.

Materials and Methods

Study Design

Six patients (two men, four women) with a mean age of 37.5 years (range: 28 to 53 years) requiring a single tooth extraction in the anterior maxilla (including premolars) were recruited. Each patient was informed about the possible risks of the study, and each provided informed written consent. The institutional ethical committee of the University of Naples approved the study protocol (154/16), and the research was conducted in accordance with the Declaration of Helsinki.

The inclusion criteria were as follows: > 18 years old; no history of systemic diseases, absence of active periodontal disease; and scheduled for a subsequent implant-supported restoration. Patients were excluded if they met the following criteria: full-mouth plaque and bleeding scores > 25%; smoking > 10 cigarettes per day; and usual contraindications to surgical procedures. Sockets were included in the study if at least 50% of the alveolar buccal bone plate was present after tooth extraction.

All selected patients underwent two surgical procedures performed by expert clinicians (Enzo V. and L.R.): (1) tooth extraction and ARP at baseline, and (2) implant placement after 12 months of alveolar healing.

Surgical Protocol

Extraction and ARP were performed at baseline using a flapless approach. Tooth extraction was performed with great care to preserve the alveolar bone plates and the surrounding soft tissues. After debridement of the socket and deep epithelialization of its gingival margin with a blade, the integrity of 50% of the buccal bone was verified with a probe. The height of the present buccal bone measured at its central portion was related to the height of the root (distance between the apex and the cementoenamel junction) minus 2 mm (mean supracrestal tissue attachment value), measured at the analog region. The socket was gently filled with DBBM (0.25- to 1-mm granules; Bio-Oss, Geistlich), avoiding excessive pressure. A CM (Mucograft, Geistlich) was shaped and adapted on the morphology of the coronal portion of the socket, then fixed with resorbable sutures (4/0 polyester, Omnia) to close the wound.

Patients underwent follow-up visits at 1, 2, 4, 8, 12, 24, and 48 weeks, with professional oral hygiene sessions every 3 months.

Twelve months after extraction and ARP, an implant (Roxolid, SLActive) was placed after elevating a full-thickness flap. The implant site was initially prepared with a trephine (external diameter of 3.5 mm, internal diameter 2.5 mm) to harvest a bone biopsy sample.

The potential to place a prosthetically guided implant without requiring further hard tissue augmentation was considered a sign of clinical efficacy of the ARP procedure. The facial bone thickness after implant placement was considered adequate if the residual facial bone thickness was ≥ 2 mm (measured with a caliper) after implant site preparation.

Before both the surgeries, patients underwent a professional oral hygiene session, and using 0.12% chlorhexidine mouthwash twice a day for 1 minute was prescribed. All surgical procedures were performed following the oral administration of...
amoxicillin (2 g) with clavulanic acids 1 hour prior and a 0.2% chlorhexidine mouthwash immediately before surgery. After both surgeries, patients were prescribed amoxicillin (1 g) with clavulanic acids twice a day for 4 days and ibuprofen (400 mg) twice a day for 2 days, all taken orally. A domiciliary prophylaxis oral hygiene protocol comprised a 0.12% chlorhexidine mouthwash twice a day, starting 24 hours after surgery and continuing for up to 8 weeks.

Control periapical radiographs were taken before and after surgical procedures, standardized using Rinn film holders.

Histologic and Histomorphometric Analyses

Biopsy samples obtained during implant surgery were stored in buffered formalin 4% and sent to a histology laboratory where they were prepared according to the methods described by Donath and Breuner. Using an abrasive rotating plate, the specimens were cut to a thickness of 60 μm. Then, slices were stained with azure II and pararosaniline (Merck). All specimens were digitalized at the same magnification using an Axio Imager M1 microscope equipped with a digital AxioCam HRc (ZEISS).

The histomorphometric analysis was performed by light microscopy using analysis software (analySIS FIVE, Olympus). The following histomorphometric parameters were evaluated as percentages of the single specimen’s total area: (1) newly formed bone tissue; (2) remnants of grafting material (both stained in dark magenta and featuring the mineralized fraction); and (3) connective tissue and bone marrow (stained in blue). For histomorphometric purposes, DBBM particles were digitally stained in green, and newly formed bone in red. The specimens were visualized at ×50, ×100, ×200, ×400, and ×630 magnifications.

Results

Clinical Results

Patient and site characteristics are reported in Table 1. The selected teeth (five central incisors and one first premolar) were scheduled for extraction due to endodontic problems or tooth fracture. All patients followed the clinical protocol and successfully completed the study (Figs 1 to 6).

After both surgeries, healing was uneventful and without infection in all patients. Soft tissue healing following ARP procedures was uneventful at 4 weeks and complete at 8 weeks.

At 12 months, all experimental sites showed a ridge dimension considered to be sufficient for the placement of a prosthetically guided implant. Implant surgery was accomplished without any bone tissue augmentation, defining a 100% clinical efficacy for ARP procedure. After flap elevation, the presence of DBBM granules was not visually detectable, and the mineralized tissue registered a high resistance to penetration with burs. This feature was not limited to just the first millimeters and was instead reported for the entire height, corresponding to the previous natural socket. All implants achieved osseointegration, and prosthetic crowns were applied.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age, y</th>
<th>Site</th>
<th>Reason for extraction</th>
<th>Type of provisional restoration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>28</td>
<td>21</td>
<td>Fracture</td>
<td>Ortho</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>31</td>
<td>11</td>
<td>Endo</td>
<td>Ortho</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>49</td>
<td>14</td>
<td>Fracture</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>29</td>
<td>11</td>
<td>Fracture</td>
<td>Ortho</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>53</td>
<td>21</td>
<td>Endo</td>
<td>Ortho + MAR</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>35</td>
<td>11</td>
<td>Fracture</td>
<td>Ortho + MAR</td>
</tr>
</tbody>
</table>

Mean 37.5

F = female; M = male; Endo = Endodontic problems; Ortho = Orthodontic; N/A – not applicable; MAR = Maryland bridge.

*FDI System.
Fig 1 (a) Clinical and (b) radiographic conditions at baseline.

Fig 2 Application of DBBM and CM in the selected site.

Fig 3 Clinical conditions at the 12-month follow-up.

Fig 4 Surgical reentry at 12 months following ARP.

Fig 5 A bone biopsy sample (internal diameter 2.5 mm) was harvested.

Fig 6 (a and b) Clinical and (c) radiographic conditions at the 6-month follow-up after prosthetic restoration.
Histologic Results

All analyzed specimens appeared well vascularized, and no signs of inflammation or CM remnants were detected (Fig 7). DBBM granules were embedded in the newly formed bone without any fibrous encapsulation and appeared connected by many thick trabecular structures. The spaces among the granules were filled with woven bone and often by lamellar bone (Figs 8 and 9). The activity of bone marrow and the areas of new bone deposition and resorption indicated an intense remodeling with anabolic activity (Fig 10). These histologic features signify an advanced stage of bone healing.

The mean ± SD percentage (range) composition of the specimen’s total area were represented as follows: 27.72% ± 5.64% (20.6% to 36.5%) newly formed bone, 29.52% ± 6.09% (19.0% to 36.6%) DBBM granules, and 42.77% ± 4.82% (34.7% to 48.2%) connective tissue and bone marrow. The mineralized fraction was 57.24% ± 4.82% (Table 2).
Discussion

ARP appears relevant for a proper implant-supported rehabilitation compensating in part for bone resorption occurring after tooth extraction, and it provides favorable conditions for implant placement.14

A retrospective volume analysis of alveolar bone remodeling showed that grafting sockets with DBBM and a resorbable barrier insertion seemed to reduce two-thirds of the negative osseous remodeling in the short term compared to that of the ungrafted sockets.20 Iorio-Siciliano et al 9 reported that at 6 months, although vertical and horizontal bone resorption occurred both in grafted and ungrafted sites, ARP using DBBM-C and CM could be recommended to reduce the collapse of the alveolar buccal wall at sites with an initial buccal wall thickness < 1 mm. In a posterior area at 6 months surgical reentry for implant placement, no significant differences were observed in dimensional changes following ARP using bovine-derived xenografts and CM compared to spontaneous healing.28

Thus, tooth loss in the anterior area represents a specific indication for ARP in order to simplify implant treatment by reducing the need for hard tissue augmentation techniques in prosthetically guided implant placement.13

As a matter of fact, Barone et al29 showed a statistically significant efficacy of ARP in avoiding further bone augmentation for prosthetically driven implant placement. Accordingly, in the present study, no sites in the anterior maxilla required any bone augmentation procedure at implant placement.

The time of alveolar bone healing after tooth extraction is not predictable,30 and grafting DBBM into the socket seems to delay such healing.21,22 In addition, there is still a lack of evidence regarding the optimal timing for implant placement following ARP.14

The present study histologically and histomorphometrically evaluated alveolar bone healing 12 months after ARP using DBBM particles covered with a xenogeneic CM in single-tooth extractions in the maxillary anterior area. The mineralized fraction represented a mean composition of 57.24% ± 4.82% of the specimen’s total area. This is in accordance with the study by Maiorana et al31 that, at 6 months, reported a smaller mineralized fraction of 47.97% ± 6.77% associated with a reduced percentage of newly formed bone. The similar percentages of residual DBBM granules (29.52% ± 6.09% in the present study and 31.97% ± 3.52% in Maiorana et al’s study) confirm that this biomaterial is a bone graft with very slow resorption. Indeed, a much more advanced stage of bone healing seems to be present at 12 months when using DBBM particles. However, the present preliminary study presents some limitations, such as the small sample size, which makes it difficult to perform a proper numerical analysis of the obtained data.

A recent similar study of ARP using collagenated DBBM covered with a CM21 showed, after 8 months of healing, a mean new bone formation of 47.52% ± 6.09% in the present study and 31.97% ± 3.52% in Maiorana et al’s study) confirming that this biomaterial is a bone graft with very slow resorption. Indeed, a much more advanced stage of bone healing seems to be present at 12 months when using DBBM particles. However, the present preliminary study presents some limitations, such as the small sample size, which makes it difficult to perform a proper numerical analysis of the obtained data.

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### Table 2 Histomorphometric Data at the 12-Month Follow-up

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Newly formed bone, %</th>
<th>DBBM, a %</th>
<th>Mineralized fraction, %</th>
<th>Connective tissue and bone marrow, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.6</td>
<td>31.2</td>
<td>51.8</td>
<td>48.2</td>
</tr>
<tr>
<td>2</td>
<td>27.5</td>
<td>27.9</td>
<td>55.4</td>
<td>44.6</td>
</tr>
<tr>
<td>3</td>
<td>36.5</td>
<td>19.0</td>
<td>55.5</td>
<td>44.5</td>
</tr>
<tr>
<td>4</td>
<td>31.5</td>
<td>33.8</td>
<td>65.3</td>
<td>34.7</td>
</tr>
<tr>
<td>5</td>
<td>23.8</td>
<td>36.6</td>
<td>60.4</td>
<td>39.6</td>
</tr>
<tr>
<td>6</td>
<td>26.4</td>
<td>28.6</td>
<td>55.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Mean</td>
<td>27.72</td>
<td>29.52</td>
<td>57.23</td>
<td>42.77</td>
</tr>
<tr>
<td>SD</td>
<td>5.64</td>
<td>6.09</td>
<td>4.82</td>
<td>4.82</td>
</tr>
</tbody>
</table>

aBio-Oss, Geistlich.
Conflicting. Nart et al33 histologically compared DBBM and DBBM-C, identifying no significant differences in the amount of newly formed bone, DBBM particles, and connective tissue. However, in other studies, DBBM-C seems to be associated with a greater amount of newly formed bone at different stages of socket healing.21,32 This observation might be explained by the reduced volume occupied by the slowly resorbable DBBM granules when the collagenated DBBM is grafted.

In the present study, the histologic examination reported a complete absence of any signs of fibrous encapsulation, and DBBM granules were connected to newly formed bone tissue often represented by lamellar bone. Compared to observations at shorter periods (4 or 6 months) following ARP with DBBM where a fibrous connective tissue is present,21,32,35–40 the present results show a highly advanced stage of alveolar bone healing at 12 months without any signs of fibrous encapsulation. Furthermore, these histologic data may explain the clinical visual absence of DBBM granules after flap elevation as well as the high resistance of mineralized tissue to bur penetration at implant placement.

Conclusions

Despite the limitations due to the small sample size, the clinical results of the present study suggest that ARP using DBBM granules covered with a CM may be considered a predictable technique that provides favorable conditions for prosthetically guided implant placement in the anterior maxilla. The histologic and histomorphometric data suggest that a 12-month period following ARP using DBBM granules may be considered adequate for implant placement if an advanced stage of alveolar bone healing is required. However, further studies are needed to evaluate these histologic and clinical features in detail.

Acknowledgments

This work was performed with institutional funding only. The authors report no conflicts of interest related to this study.

References


