Received: 28 June 2023 | Revised: 30 November 2023 | Accepted: 16 December 2023

DOI: 10.1111/clr.14235

ORIGINAL ARTICLE

CLINICAL ORAL IMPLANTS RESEARCH WILEY

Histological and radiological outcome after horizontal guided bone regeneration with bovine bone mineral alone or in combination with bone in edentulous atrophic maxilla: A randomized controlled trial

Hanna Aludden[1,2] | Thomas Starch-Jensen[3,4] | Christer Dahlin[1,2] | Joana Sdik[5] | Andreas Cederlund[6] | Arne Mordenfeld[7]

[1]Department of Biomaterials, BIOMATCELL VINN Excellence Center, Institute for Surgical Science, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[2]Department of Oral and Maxillofacial Surgery, NU-Hospital, Organization Trollhättan, Trollhättan, Sweden

[3]Department of Oral and Maxillofacial Surgery, Aalborg University Hospital, Aalborg, Denmark

[4]Department of Clinical Medicine, The Faculty of Medicine, Aalborg University, Aalborg, Denmark

[5]Private Practice, Stockholm, Sweden

[6]Department of Oral Radiology, Eastman Institute, Stockholm County Council, Region Stockholm, Stockholm, Sweden

[7]Plastic and Oral & Maxillofacial Surgery, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

Correspondence
Hanna Aludden, Department of Oral and Maxillofacial Surgery, NU-Hospital, Organization Trollhättan; Department of Biomaterials, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
Email: hanna.aludden@vgregion.se

Funding information
Geistlich Pharma

Abstract

Objective: To assess the radiological and histological outcome after horizontal guided bone regeneration (GBR) with deproteinized bovine bone mineral (DBBM) alone or in combination with particulate autogenous bone (PAB).

Materials and Methods: Eighteen edentulous patients with an alveolar ridge of ≤4 mm were included in this split-mouth randomized controlled trial. Horizontal GBR with a graft composition of 100% DBBM (100:0) on one side and 90% DBBM and 10% PAB (90:10) on the other side were conducted in all patients. Cone beam computed tomography (CBCT) was obtained preoperatively, immediately postoperative, and after 10 months of healing. Width and volumetric changes in the alveolar process were measured on CBCT. Implants were placed after 10 months of graft healing where biopsies were obtained for histomorphometrical evaluation.

Results: The gained widths were 4.9 (±2.4) mm (100:0) and 4.5 (±2.0) mm (90:10) at 3 mm from the top of the crest, and 5.6 (±1.3) mm (100:0) and 4.6 (±2.1) mm (90:10) at 6 mm from the top of the crest. The mean volumetric reductions were 32.8% (±23.8) (100:0) and 38.2% (±23.2) (90:10). Histomorphometry revealed that mean percentages of bone were 50.8% (±10.7) (100:0) and 46.4% (±11.3) (90:10), DBBM were 31.6% (±12.6) (100:0) and 35.4% (±14.8) (90:10), and non-mineralized tissue were 17.6% (±11.7; 100:0) and 18.2% (±18.2) (90:10). No significant differences were evident between in any evaluated parameters.

Conclusions: There were no additional effects of adding PAB to DBBM regarding bone formation, width changes, or volumetric changes after 10 months of graft healing.

KEYWORDS
3D imaging, alveolar ridge augmentation, bone substitutes, bone transplantation, dental implants, guided bone regeneration

1 | INTRODUCTION

Horizontal reconstruction with bone substitutes alone or in combination with particulate autogenous bone (PAB) according to the principles of guided bone regeneration (GBR) is a well-documented method to enhance the width of the alveolar process prior to implant placement (Berglundh et al., 2002; Block et al., 2012; Friedmann et al., 2002; Hellem et al., 2003; Meloni et al., 2017; Mendoza-Azpur et al., 2019; Mordenfeld et al., 2014, 2017; Urban et al., 2011, 2013).

The GBR technique using a particulate graft requires a graft material that allows the ingrowth of new bone, maintains the volume, and provides osteoconductive properties of the graft even during pressure from the oral mucosa, lips, and cheeks. Deproteinized bovine bone mineral (DBBM) is a xenograft that is biocompatible and osteoconductive bone substitute. Long-term studies of the material have indicated no or limited resorption (Mordenfeld et al., 2010). PAB is commonly combined with DBBM in different percentages to improve the osteogenic potential of the graft. The most common particulate graft material composition for GBR is a 50:50 mix of PAB and DBBM and has been the suggested graft composition when augmenting extensive defects with a fixed membrane (Meloni et al., 2017; Urban et al., 2011, 2013). However, a preclinical study in minipigs revealed that the addition of PAB to DBBM did not have a positive effect on the regenerative outcome or the width changes of the graft after horizontal GBR (Aludden et al., 2017, 2020, 2021). Furthermore, a preclinical study comparing a 50:50 mix of PAB and DBBM to 100:0 DBBM in horizontal defects revealed an identical conclusion (Song et al., 2021). Similar findings have been presented in conjunction with maxillary sinus floor augmentation (Pikdoken et al., 2011).

For the reconstruction of edentulous jaws, iliac bone graft has commonly been used due to the large amount of graft material needed (Dasmah et al., 2012; Nyström et al., 2009; Sbordone et al., 2012). However, this approach requires a second donor site with additional morbidity, extended operation time, complex surgical skills, and higher costs. Nevertheless, a previous randomized controlled trial augmented totally and partially edentulous jaws according to the GBR principles, with two different ratios of DBBM and PAB (60:40 and 90:10; Mordenfeld et al., 2014). This study reported no histomorphometric differences between the two compositions after 9 months of healing but significantly lower width reduction for the 60:40 mixture, which did not have an apparent clinical impact on implant installation since the difference was only 0.7 mm (Mordenfeld et al., 2014). To expand on these findings, the present study aims to investigate the effect of removing PAB from the graft, that is, 100:0 DBBM:PAB compared with the previously investigated ratio of 90:10 DBBM:PAB by histological and radiological evaluation in a randomized controlled trial. The null hypothesis is no statistically significant difference between the two graft compositions.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

The present randomized controlled trial was designed as a split-mouth study and was registered at ClinicalTrial.gov with ID number NCT04755166. The study was approved by the North Denmark Region Committee on Health Research Ethics (approval number: N-20160035) and the Swedish Committee on Health Research Ethics Uppsala (approval number: 2015/525). The study conforms to the declaration of Helsinki.

The study population included patients with an edentulous maxilla in need of horizontal augmentation of the alveolar process prior to implant placement.

The alveolar ridge was classified according to the Cawood classification in each patient (Cawood & Howell, 1988). In most cases, the anterior part of the maxilla was class IV, and in some cases, class V. In the posterior part of the maxilla, all cases were class V or VI. Inclusion and exclusion criteria are outlined in Table 1.

All patients were required to read, understand, and sign an informed consent before inclusion. To remain in the study, it was mandatory to follow all postoperative restrictions and attend all scheduled follow-up appointments.

The patients were recruited from the Department of Oral and Maxillofacial Surgery, Aalborg University Hospital, Denmark, and the Department of Oral and Maxillofacial Surgery, Gävle County Hospital, Sweden, from 2015 to 2018. A total of 18 patients (7 men and 11 women) with a mean age of 65 years (range 39–83) were included in the study. All 18 patients underwent bilateral horizontal GBR. In 15 patients, bilateral maxillary sinus floor elevation was performed in conjunction with horizontal GBR due to a lack of adequate vertical bone height in the posterior maxillary region. All patients had written and oral information about the postoperative restrictions, which included no use of the prosthesis for 2 weeks after horizontal GBR, followed by soft reline of the prosthesis during the entire healing period of 10 months.

TABLE 1 Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Adult patients (>20 years) • Edentulous maxilla • A width of the alveolar process of <4.5 mm (measured on CBCT at 3 mm from the top of the crest) • Cawood classification IV or V in the anterior part of the maxilla	• Smoking • Uncontrolled systematic disease • Previous history of irradiation to the area of treatment

Abbreviation: CBCT, Cone Beam Computer Tomography.

All patients were allocated to a number for randomization and for blinding during the analyses. The allocation sequence of the grafting procedure was computer generated by a statistician and concealed in envelopes until the moment of randomization during surgery. An envelope marked with the number of the patient was opened during surgery and allocated a graft composition to the right or the left side.

2.2 | Horizontal guided bone regeneration

All procedures were performed by three experienced surgeons (AM, TSJ, and HA) under either local anesthesia in combination with oral sedation (midazolam, 5–10 mg) or general anesthesia. As a prophylactic measure, all patients received V-penicillin (1 g × 3) for 7 days starting 1 day preoperatively; in the case of allergy, clindamycin (300 g × 3) was given. After a crestal incision with bilateral releasing incisions, the mucoperiosteal flap was elevated and reflected bilaterally to expose the buccal and palatal aspects of the edentulous area (Figure 1a). The cortical bone was perforated with a small round bur. The mucoperiosteal flap was elongated through an incision of the periosteum from one releasing incision to the other. Autogenous bone was locally harvested with a bone scraper. DBBM particles (0.25–1 mm size, Bio-Oss, Geistlich Pharmaceutical) were mixed by weight with PAB in a 90:10 ratio (mean 1.0 g DBBM: 0.13 g PAB), or used without mixing in a 100:0 ratio (1.0 g DBBM). Both graft compositions were mixed with autogenous blood from the surgical site or blood collected from a vein in the hand or foot when a sufficient amount could not be achieved from the surgical site.

Left or right side was randomized to receive a graft composed of DBBM:PAB in a ratio of 100:0 or 90:10. The grafts were placed on the buccal aspect of the alveolar process from the region of the central incisor to the region of the second premolar (Figure 1b), densely packed under a resorbable collagen membrane (Bio-Gide, Geistlich Pharmaceutical) fixed by titanium pins (Master Pin). Two pins were placed at the palatine aspect and 2–3 pins on the buccal aspect (Figure 1c). The intention was to create a width of the ridge of 8–10 mm. The incisions were closed by horizontal mattress sutures as well as single interrupted and continuous resorbable sutures (4-0 Vicryl).

The patients were not allowed to wear their dentures for 14 days at which time the facial aspect of the dentures was removed and relined with a soft material so that minimal of the soft material was in contact with the augmented area. Patients were instructed to rinse with chlorhexidine 0.1% twice a day until suture removal. The sutures were removed after 10–14 days, and the patients were followed clinically after 1 and 2 months and checked for complications.

2.2.1 | Implant installation and biopsy retrieval

After 10 months, a second surgery for implant installation and biopsy retrieval was performed. A single dose of amoxicillin (2 g) was administered 1 h preoperatively. After a crestal incision with bilateral releasing incisions, the mucoperiosteal flap was elevated bilaterally to expose the augmented area (Figure 1d).

Two biopsies, one from each side, were retrieved with a trephine bur (Ø 2.6/3.3 mm) in the approximate region of the first premolar and perpendicular to the buccal surface (Figure 2). To ensure that the whole width of the transplant and the lateral aspect of the host bone were retrieved, the trephine bur was inserted until the medial aspect of the alveolar crest was reached. To prevent overheating, the procedure was performed during constant irrigation with saline. The specimens were immediately fixed in formalin. Three implants with a diameter of 3.6 or 4.2 mm (Astra EV) were placed on each side. All implants could be placed with a variation in the grade of primary stability. All implants had cover screws and the incisions were closed by horizontal mattress sutures as well as single interrupted and continuous resorbable sutures (4-0 Vicryl). The patients were allowed to use their dentures immediately after surgery. Three months after implant placement, a third surgery was performed, and healing abutments were mounted. All dentures were adjusted by the prosthetic dentist and a fixed prosthetic solution was produced by the same prosthodontist after implant healing.

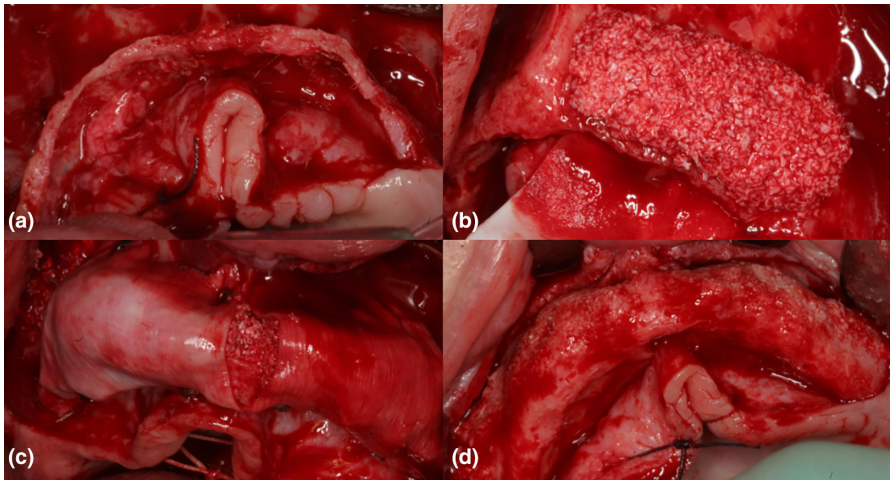

FIGURE 1 (a) Exposed maxilla preoperative. (b) Graft material placed on the lateral aspect of the alveolar process. (c) Graft material densely packed under a resorbable collagen membrane fixed with titanium pins. (d) Exposed maxilla after 10 months of graft healing.

2.3 | Radiographic examination with width and volumetric assessment

Cone beam computed tomography (CBCT; i-CAT Cone Beam 3D Imaging System, Imaging Sciences International Inc.) with voxel spacing of 0.4 × 0.4 × 0.4 mm of the maxilla was obtained preoperatively, immediately postoperatively, and after 10 months of healing (1 week before implant installation). All width measurements were made on tomographic slices perpendicular to the longitudinal axis of the alveolar crest using computer software (ITK-SNAP; Yushkevich et al., 2006), 3 and 6 mm from the top of the crest, at two different sites on each side, at the expected implant sites for the two most anterior implants on each side. In the midline of the jaw, on the axial view, an anatomic reproducible landmark (the incisal foramen and the anterior nasal spine) was defined and a straight line was drawn through it. The distance from the measuring point to this line, at the middle of the alveolar crest, was obtained using the software ruler. The same anatomic landmarks and distances were used for measurements on CBCT scans immediately after grafting and 10 months after the grafting procedure. The width of the residual bone and the total width (host bone and graft material) were obtained from the CBCT scans immediately after grafting, as the graft could easily be distinguished from the host bone. On the scans 10 months after grafting, only the total width (host bone and transplant) was measured since it was difficult to distinguish the host bone from the graft in some cases (Figure 3a–d). The graft width after 10 months was calculated by subtracting the width of the host bone, which was obtained from the scans immediately after grafting, from the total width at 10 months.

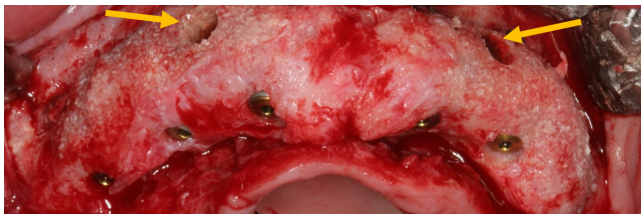

FIGURE 2 Illustration of bone biopsies from the two graft compositions.

Volumetric measures were performed using semiautomatic segmentation in ITK-SNAP software (Yushkevich et al., 2006). In postoperative axial images, the most distal border of the transplant in the maxillary arch was identified and marked with a horizontal (frontal) line. The distance from the anterior nasal spine to the horizontal line was obtained. This distance was transferred to the preoperative scan and the 10-month postoperative scan to define the same area for measuring the volume of the maxilla. The anterior nasal spine was set as the superior border of the measured volumes. The total volume for the right (shown in red) and left side (shown in green) (Figure 4a–c) of the maxilla was measured by marking the area in every fifth axial image starting from the maxillary crest to the superior border of the anterior nasal spine. Bone and transplant volumes were calculated by the software, with results presented in cubic millimeters (mm^3). The change in transplant volume for each side of the maxilla and between the different CBCT examinations was

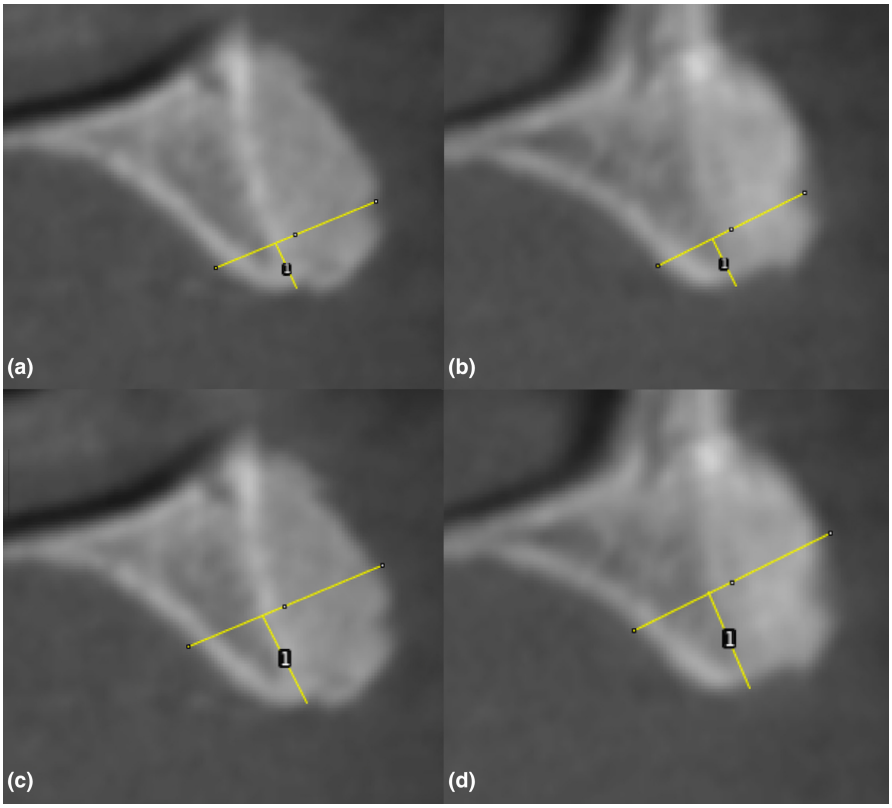

FIGURE 3 (a) Width measurements postoperatively 3 mm from the top of the crest. (b) Width measurements after 10 months of graft healing 3 mm from the top of the crest. (c) Width measurements postoperatively 6 mm from the top of the crest. (d) Width measurements after 10 months of graft healing 6 mm from the top of the crest.

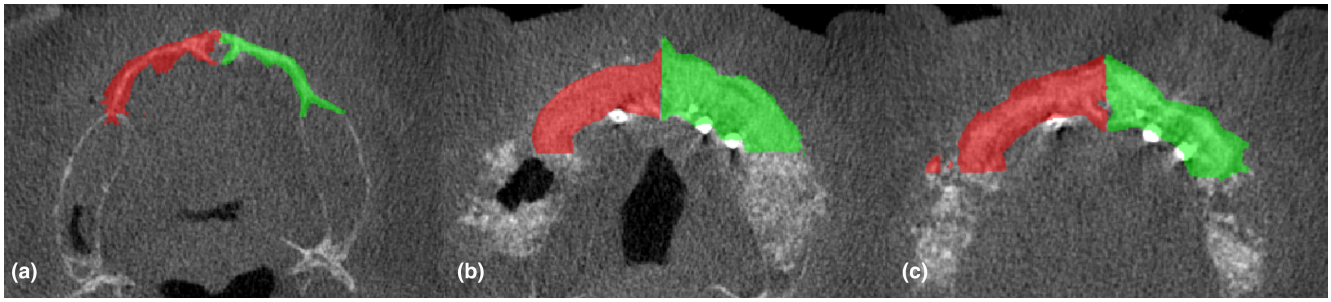

FIGURE 4 (a) Volumetric measurements preoperatively. (b) Volumetric measurements postoperatively. (c) Volumetric measurements after 10 months of graft healing.

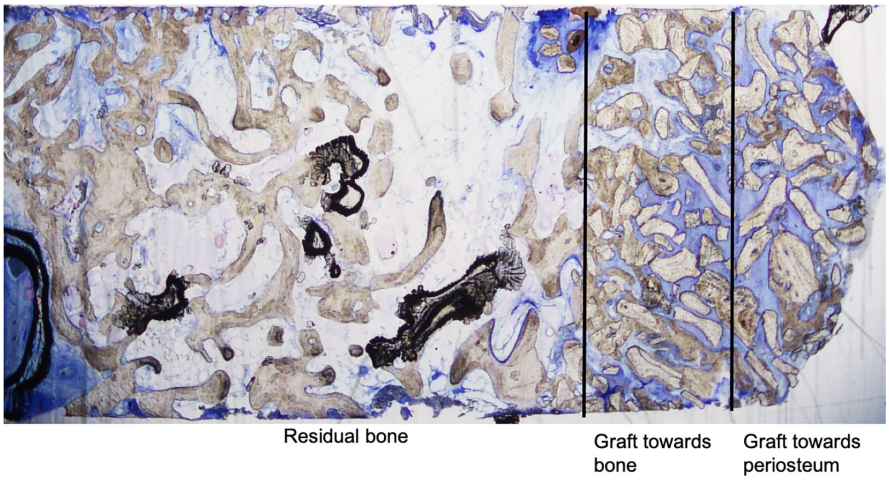

FIGURE 5 Illustration of a histological specimen divided into two parts (i.e., toward the host bone and the periosteum).

calculated for each patient using the preoperative examination as a reference.

2.4 | Histologic preparation and assessment

The specimens were rinsed in water and treated by stepwise dehydration in a graded ethanol series, followed by embedding in plastic resin (LR White, London Resin Co. Ltd). The samples were divided in the long axis of the biopsy and mounted on supportive plexiglass slides. A 10- to 15-μm-thick central ground section was prepared from each specimen (EXAKT1 Apparatebau GmbH & Co; Donath & Breuner, 1982) and stained with toluidine blue.

Quantitative histomorphometry was performed using light optical microscopy (Nikon Eclipse E600). All measurements were performed manually on the computer screen connected to a microscope in Nikon NIS elements software. A 10× magnification was used. The same researcher (HA) performed all measurements.

Each biopsy consisted of host bone medially and the grafted area laterally. The border laterally was the periosteum. The grafted area was identified and distinguished from the host bone. Superiorly and inferiorly, the specimen was restricted by means of the size of the trephine.

The specimen was divided into two equal segments, one medial facing the host bone and one facing the lateral aspect of the grafted area. Hence, two ROI: s (medial and lateral, respectively) were analyzed (Figure 5).

In case of loose particles laterally, they were included in the lateral ROI.

The following histomorphometric measurements were performed in all specimens:

1. Total area of the grafted area, measured in both the segment facing the host bone and the segment facing the lateral aspect of the graft
2. Total bone area, measured in both the segment facing the host bone and the segment facing the lateral aspect of the grafted area.
3. Total area of DBBM, measured in both the segment facing the host bone and the segment facing the lateral aspect of the grafted area.
4. Total area of non-mineralized tissue (NMT), measured in both the segment facing the host bone and the segment facing the lateral aspect of the grafted area.

2.5 | Statistical analyses

A sample size calculation was conducted using Stata 16.1 (Stata Corp P). Based on an alpha significance level of 0.05, a total of 18 patients

were needed to achieve 80% power to detect a difference of 1 mm (SD 1.5) in width changes between the two groups (100:0 and 90:10).

We consider 1 mm as clinically meaningful. Hence, this number was considered relevant for the present study.

Data management and statistical analyses including the calculation of descriptive statistics were conducted using R version 2.8.0 (R Development Core Team, 2008). The primary outcomes were changes in the width, changes in volume, and percentage of bone in the grafts. Mean values and standard deviation (SD) were reported for each type of material. The two graft compositions were compared using a paired *T*-test. Assumptions of normality were visually inspected by histograms and QQ plots. Two-tailed *p*-values <.05 were considered statistically significant.

3 | RESULTS

Of 36 grafts, 33 healed uneventfully, whereas three sites presented with mucosal dehiscence (two in the 100:0 group and one in the 90:10 group). One patient was treated with antibiotics in combination with chlorhexidine and the other two only with chlorhexidine. All sites healed within 4 weeks. One patient was excluded from the analysis due to a lack of cooperation with the postoperative rules. Hence, a total of 17 patients were included after 10 months of healing. Specimens from all included patients could be used for histomorphometric analyses as well as for volumetric analyses. For the width measurements, 15 patients were assessed due to difficulties in distinguishing the host bone from the graft in two patients, which were considered to have a high risk of bias.

3.1 | Radiographic results

Width and volumetric changes after 10 months varied widely across individuals. Width changes of the grafts are presented as mean values with standard deviation in Tables 2 and 3. For the two compositions, the mean gains in width at 3 mm from the top of the crest were 4.9 mm (100:0) and 4.5 mm (90:10). At 6 mm from the top of the crest, the mean gains were 5.6 mm (100:0) and 4.6 mm (90:10). There was no statistically significant difference between the two compositions ($p > .30$). Ten months after surgery, the width reduction of the alveolar process was 29.0% (100:0) and 21.1% (90:10) at 3 mm from the top of the crest, and 19.1% (100:0) and 18.7% (90:10) at 6 mm from the top of the crest. There were no statistically significant differences in width reductions between the two compositions ($p > .30$).

Likewise, there were no significant differences regarding gained width or width reduction between the 100:0 and 90:10 mixtures at 3 mm or 6 mm from the top of the crest (both $p > .30$). However, the gain in width was significantly increased with a thinner host bone ($p < .05$). The volumetric changes are presented in Table 4. The mean volumetric reduction was 32.9% for the 100:0 graft and 39.4% for the 90:10 graft. There was no significant difference in volume reduction between the two graft compositions ($p > .30$).

3.2 | Qualitative observations of the histological sections

The bone biopsies differed in length, depending on the width of the graft and the width of the host bone. DBBM particles were easily recognized and frequently present, while PAB particles were not. Irrespective of graft composition, no major qualitative differences were observed. All biopsies demonstrated bone and soft tissue integration of DBBM particles in various degrees, but more frequently, the DBBM particles appeared to be encapsulated by bone. However, in some sections, quite large regions of NMT, formed in close vicinity to DBBM particles, could be observed. These formations seemed to contain small fragments of DBBM particles. Macrophages and plasma cells were observed in these NMT regions. Inflammatory cells were commonly observed irrespective of sample. No qualitative differences could be detected between the bone encapsulation of the particles located toward the host bone or particles located toward the periosteum. A few PAB particles

TABLE 2 Dimensions at 3 mm from the top of the crest.

	Preoperative	After grafting	After 10 months	Gained width at 10 months	Reduction of the graft
Mixture	Mean (SD) mm	Mean (SD) mm	Mean (SD) mm	Mean (SD) mm	Mean (SD) %
100:0	4.1 (1.2)	10.8 (1.9)	9.0 (2.0)	4.9 (2.4)	29.0 (28.6)
95% CI	2.2, 4.4	5.8, 7.6	5.7, 10.6	3.5, 6.2	−44.8, −13.2
90:10	3.9 (1.1)	9.9 (1.0)	8.4 (1.7)	4.5 (2.0)	21.1 (38.8)
95% CI	2.2, 4.4	5.4, 6.5	5.7, 10.0	3.5, 5.6	−42.5, 0.4
Difference	0.2	0.9	0.6	0.4	7.9
95% CI	−0.97, 0.52	−1.95, 0.43	−1.31, 0.66	−1.1, 0.58	−5.72, 21.59
p-value	>.300	.200	>.300	>.300	>.300

Abbreviations: CI, 95% confidence interval; SD, standard deviation; 100:0, 100% deproteinized bovine bone mineral; 90:10, 90% deproteinized bovine bone mineral; 10% particulate autogenous bone.

TABLE 3 Dimensions at 6 mm from the top of the crest.

	Preoperative	After grafting	After 10 months	Gained width at 10 months	Reduction of the graft
Mixture	Mean (SD) mm	Mean (SD) mm	Mean (SD) mm	Mean (SD) mm	Mean (SD) %
100:0	5.4 (1.6)	12.3 (1.1)	11.0 (1.2)	5.6 (1.29)	19.1 (14.9)
95% CI	3.8, 7.1	6.3, 7.5	8.7, 13.4	4.9, 6.3	−27.3, −10.8
90:10	5.1 (1.8)	10.7 (2.0)	9.7 (2.0)	4.6 (2.1)	18.7 (21.5)
95% CI	4.1, 6.1	4.5, 6.7	7.6, 11.7	3.5, 5.6	−30.6, −6.8
Difference	0.3	1.6	1.3	1.0	0.4
95% CI	−0.82, 1.07	−2.40, −0.35	−1.85, −0.24	−1.8, −0.18	−6.22, 6.89
p-value	>.300	.013	.016	.016	>.300

Abbreviations: CI, 95% confidence interval; SD, Standard deviation; 100:0, 100% deproteinized bovine bone mineral; 90:10, 90% deproteinized bovine bone mineral; 10% particulate autogenous bone.

TABLE 4 Graft volume 10 months after augmentation.

Mixture	Postoperative (SD) mm^3	After 10 months (SD) mm^3	Width reduction (SD) %
100:0	1499 (407)	1006 (431)	32.9 (23.8)
95% CI	1273, 1725	767, 1245	−46.1, −19,7
90:10	1640 (432)	1014 (512)	39.4 (23.2)
95% CI	1401, 1880	730, 1298	−52.3, −26.6
Difference	141	8	6.5
95% CI	−73.3, 355.7	−164.2, 180.0	−16.8, 3.8
p-value	.18	>.3	.21

Abbreviations: CI, 95% confidence interval; SD, standard deviation; 100:0, 100% deproteinized bovine bone mineral; 90:10, 90% deproteinized bovine bone mineral; 10% particulate autogenous bone.

were observed and they appeared to be lighter stained and had empty osteocyte lacunae. They were often surrounded by NFB with large osteocytes.

3.3 | Histomorphometric results

Thirty-four specimens, 17 from each grafted site, were histomorphometrically evaluated. Two specimens could not be evaluated due to artifacts after the histological preparation. The grafted tissue was clearly distinguished from the residual bone. No effort was made to distinguish the grafted PAB particles from the newly formed bone since these were sparse and generally resorbed. The results from the histomorphometric analyses are presented in Table 5.

Across all results, there was a wide individual range. For the two compositions, the mean percentages of bone were 50.8% (100:0) and 46.4% (90:10). The mean percentages of DBBM were 31.6% (100:0) and 35.4% (90:10), while the mean percentages of NMT were 17.6% (100:0) and 18.2% (90:10). There were no statistically significant differences between the two graft compositions regarding percentage of bone, NMT, or DBBM. When comparing the part of the graft located toward the host bone and the part of the graft located toward the periosteum, there was no statistically significant difference; neither between the two locations within the graft nor between the two graft compositions.

4 | DISCUSSION

In this randomized controlled trial, histological and radiological evaluation after horizontal GBR revealed no statistically significant differences between grafts containing two different ratios of DBBM and PAB (100:0 and 90:10) regarding changes in width, volume, or bone regeneration. The stability of the graft during the consolidation period in terms of fixation of the membrane is an important factor for the regenerative outcome after horizontal GBR (Mir-Mari et al., 2016, 2017). Even if collagen membranes undergo degradation, the organization and induction in bone formation following GBR have already taken place if the graft is stable during the initial healing period. It has previously been assumed that difficulty in controlling the stability of the grafted particles produces a relatively high percentage of soft tissue ingrowth instead of new bone (Artzi et al., 2004). Clinical studies have demonstrated a superior increase in the width of the alveolar process when the graft material was stabilized under a fixed resorbable collagen membrane using titanium pins (Mendoza-Azpur et al., 2019; Urban et al., 2011, 2013)

TABLE 5 Histomorphometric results 10 months after augmentation.

Graft composition	Overall			Toward host bone			Toward periosteum		
	Bone (SD)	DBBM (SD)	NMT (SD)	Bone (SD)	DBBM (SD)	NMT (SD)	Bone (SD)	DBBM (SD)	NMT (SD)
100:0	50.8% (10.7)	31.6% (12.6)	17.6% (11.7)	52.4% (11.0)	32.4% (11.8)	15.22% (11.7)	50.0% (7.4)	36.8% (9.92)	13.2% (7.5)
90:10	46.4% (11.3)	35.4% (14.8)	18.2% (12.6)	50.1% (10.6)	33.9% (14.9)	33.9% (14.9)	47.6 (14.25)	37.0% (12.4)	15.4% (11.5)
Difference	4.4	3.8	0.6	2.3	1.5	18.7	2.4	0.2	2.2
95% CI	−1.1, 0.58	−1.8, 0.44	−3.2, 4.8	−0.85, 1.2	−2.5, 3.0	−6.6, 5.4	−1.85, 0.24	−2.3, 0.17	−5.53, 10.1
p-value	.06	.23	>.3	>.3	>.3	>.05	>.3	>.3	>.3

Abbreviations: CI, 95% confidence interval; DBBM, deproteinized bovine bone mineral; NMT, non-mineralized tissue; SD, standard deviation; 100:0, 100% DBBM; 90:10, 90% DBBM; 10% particulate autogenous bone.

compared to no fixation of the membrane (Mordenfeld et al., 2014, 2017). Similar findings have been presented in a radiological experimental study (Mir-Mari et al., 2016, 2017; Stavropoulos et al., 2004). A firm and stable graft will probably counteract the compression of the augmented area, which may be a more important factor than the composition of the graft (i.e., relative amounts of DBBM and PAB) for horizontal GBR; therefore, this approach was chosen in the present study.

Hammerle et al. have concluded that the combination of DBBM and a collagen membrane is an effective treatment option for horizontal bone augmentation after a healing period of 9–10 months, before implant placement (Hämmerle et al., 2008). Since that, several studies have presented satisfying results with a healing period of 8–10 months after augmenting horizontal defects using DBBM with different compositions of PAB (Friedmann et al., 2002; Mendoza-Azpur et al., 2019; Mordenfeld et al., 2014; Urban et al., 2011, 2013). In a previous study augmenting edentulous jaws, loose DBBM particles were noticed at surgical reentry after 8 months (Mordenfeld et al., 2014). Hence, an extended healing time of 10 months was chosen for the present study to ensure mineralized bone even at the lateral aspect of the augmented area.

After 10 months of healing, no statistically significant differences were found in bone formation, neither between the two graft compositions nor between the inner and the outer areas of the grafts.

This study demonstrated gained widths of 4.9 mm (100:0) and 4.5 mm (90:10) at 3 mm from the top of the crest. These values are slightly lower compared to those previously reported in clinical studies assessing fixed grafts, which varied between 5.0 and 5.7 mm (Mendoza-Azpur et al., 2019; Urban et al., 2011, 2013). However, the gained width at 6 mm from the top of the crest was 5.6 for the 100:0 graft, which is comparable to previous reports (Mendoza-Azpur et al., 2019; Urban et al., 2011, 2013). It is always a challenge to compare results across different studies since the vertical position of the measurements is important. The coronal part of the graft maintains the width to a reduced degree compared to the apical part (Mir-Mari et al., 2016, 2017). This means that the result is improved if the measurements are performed only a few millimeters more apically. Different healing periods could also affect the result. If the graft is not completely integrated, parts of the superficial layer might be elevated with the flap and the radiographic appearance might not be in accordance with the clinical situation. However, if the width of the alveolar process allows implant installation with a sufficient diameter of the implant and with preservation of the buccal bone of at least 1.5 mm, which has been reported as a statistically significant value for reducing peri-implant complications (Monje et al., 2019), the gained width value might not be that important.

In this study, two different vertical positions (3 and 6 mm from the top of the crest) were chosen for width measurements, a method previously applied for the horizontal GBR of edentulous jaws (Mordenfeld et al., 2014). Mordenfeld et al. presented an improved preservation of the width 3 mm from the top of the crest using grafts containing a higher percentage of PAB (Mordenfeld et al., 2014). This contrasts with the present study, where addition of PAB was not

favorable compared to no addition of PAB, neither at 3 mm nor 6 mm from the top of the crest. The similar results for the two grafts could be explained by the small difference in the relative amount of PAB (10%), between the two compositions, the extended healing period from 8 to 10 months, and fixation of the membrane with pins.

Reports of volumetric evaluation by three-dimensional measurements after horizontal GBR are limited in the literature (Dasmah et al., 2012; Mordenfeld et al., 2014). A systematic review has reported that DBBM alone reduced the resorption of the augmented region compared with compositions featuring different ratios of DBBM and PAB (Elnayef et al., 2018). This finding has been confirmed in a preclinical study assessing volumetric changes in grafts with different compositions of DBBM and PAB, where volumetric stability was superior with 100:0 DBBM (Aludden et al., 2021). Three-dimensional measurements in a clinical study revealed a volume reduction of 55.3% using a graft with a 90:10 composition of DBBM and PAB, and 53.8% with a 60:40 composition after 8 months of graft healing; there were no significant differences between the compositions (Mordenfeld et al., 2014). This is indicative of more extensive resorption than in the present study, which showed volume reductions of 39.4% (90:10) and 32.9% (100:0). The observed differences could be explained by the fixation of the graft, which was not used by Mordenfeld et al. but seems important to maintain the stability of the graft and might be an important factor to maintain volume of the graft than the composition of the graft material.

New bone formation into the grafted area can occur both from the host bone if properly perforated and from the periosteum (Gordh et al., 1997; Ortak et al., 2005). During the histomorphometric analyses in this study, the biopsy was divided into two (i.e., the part closest to the host bone and the part closest to the soft tissue) to evaluate if there was a difference. This approach was previously used by Mordenfeld et al., who observed more bone formation close to the host bone compared to the portion close to the periosteum.

It has previously been advocated that an extended healing period of the graft could increase the ingrowth of newly formed bone into the graft closer to the periosteum (Araújo et al., 2002). In a preclinical study investigating the pattern of in-growth of mineralized bone into particulate grafts, mineralization was gradually seen during the healing process and the consolidation period appeared to have an impact on the mineralization of the graft (Aludden et al., 2020).

The healing period of 10 months is relatively long and could be the reason for no difference between the two graft compositions since it is previously known that the osteoinductive effect of autogenous bone is important in the early healing phase (Jensen et al., 2006). According to the present study design, it is not possible to investigate this early effect of the addition of PAB which could be considered as a limitation.

In future studies, it could be an advantage to evaluate the graft after different healing periods, including the early healing phase.

Within the limitations of the present study, it seems like DBBM alone does not negatively impact new bone formation and performs similarly to PAB-containing grafts in terms of width and volume maintenance of the alveolar process after 10 months of healing.

Hence, it appears that horizontal GBR with DBBM alone could be used as a graft material in horizontally resorbed edentulous jaws to provide sufficient ridge dimensions for implant placement after a graft healing of 10 months. However, further randomized controlled trials with a larger number of individuals and long-term observation periods are needed before definite conclusions can be drawn regarding the performance of the grafts.

5 | CONCLUSION

There were no additional effects of adding PAB to DBBM regarding bone formation, width changes, or volumetric changes after 10 months of graft healing following horizontal augmentation by the GBR principles in edentulous atrophic maxilla.

AUTHOR CONTRIBUTIONS

AM and HA conceived the ideas. HA, AM, and TJ planned and participated in the surgeries. HA and JS collected the data. HA, AM, CD, and AC analyzed the data. AC developed the method of radiological analyses. HA and AM led the writing. All authors contributed to the manuscript.

ACKNOWLEDGMENTS

Bio-Oss and Bio-Gide membranes were kindly provided by Geistlich Pharma AG, Wolhusen, Switzerland.

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

Aludden, H., Dahlin, A., Starch-Jensen, T., Dahlin, C., & Mordenfeld, A. (2017). Histomorphometric analyses of area fraction of different ratios of bio-Oss(R) and bone prior to grafting procedures—An in vitro study to demonstrate a baseline. *Clinical Oral Implants Research*, *29*, 185–191. https://doi.org/10.1111/clr.13079

Aludden, H., Mordenfeld, A., Cederlund, A., Dahlin, C., Spin-Neto, R., Veiss-Pedersen, P., & Starch-Jensen, T. (2021). Radiographic changes in height and volume after lateral GBR procedures with different ratios of deproteinized bovine bone mineral and autogenous bone at different time points. An experimental study. *Clinical Oral Implants Research*, *32*(2), 167–179. https://doi.org/10.1111/clr.13687

Aludden, H., Mordenfeld, A., Dahlin, C., Hallman, M., & Starch-Jensen, T. (2020). Histological and histomorphometrical outcome after lateral guided bone regeneration augmentation of the mandible with different ratios of deproteinized bovine bone mineral and autogenous bone. A preclinical in vivo study. *Clinical Oral Implants Research*, *31*(10), 1025–1036. https://doi.org/10.1111/clr.13649

Araújo, M. G., Sonohara, M., Hayacibara, R., Cardaropoli, G., & Lindhe, J. (2002). Lateral ridge augmentation by the use of grafts comprised

of autologous bone or a biomaterial. An experiment in the dog. *Journal of Clinical Periodontology*, *29*(12), 1122–1131. https://doi.org/10.1034/j.1600-051x.2002.291213.x

Artzi, Z., Weinreb, M., Givol, N., Rohrer, M. D., Nemcovsky, C. E., Prasad, H. S., & Tal, H. (2004). Biomaterial resorption rate and healing site morphology of inorganic bovine bone and beta-tricalcium phosphate in the canine: A 24-month longitudinal histologic study and morphometric analysis. *The International Journal of Oral & Maxillofacial Implants*, *19*(3), 357–368.

Berglundh, T., Persson, L., & Klinge, B. (2002). A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. *Journal of Clinical Periodontology*, *29*(Suppl 3), 197–212. discussion 232–193.

Block, M. S., Ducote, C. W., & Mercante, D. E. (2012). Horizontal augmentation of thin maxillary ridge with bovine particulate xenograft is stable during 500 days of follow-up: Preliminary results of 12 consecutive patients. *Journal of Oral and Maxillofacial Surgery*, *70*(6), 1321–1330. https://doi.org/10.1016/j.joms.2012.01.026

Cawood, J. I., & Howell, R. A. (1988). A classification of the edentulous jaw. *International Journal of Oral and Maxillofacial Surgery*, *17*(4), 232–236. https://doi.org/10.1016/s0901-5027(88)80047-x

Dasmah, A., Thor, A., Ekestubbe, A., Sennerby, L., & Rasmusson, L. (2012). Particulate vs. block bone grafts: Three-dimensional changes in graft volume after reconstruction of the atrophic maxilla, a 2-year radiographic follow-up. *Journal of Cranio-Maxillo-Facial Surgery*, *40*(8), 654–659. https://doi.org/10.1016/j.jcms.2011.10.032

Donath, K., & Breuner, G. (1982). A method for the study of undecalcified bones and teeth with attached soft tissues. The sage-Schliff (sawing and grinding) technique. *Journal of Oral Pathology*, *11*(4), 318–326. https://doi.org/10.1111/j.1600-0714.1982.tb00172.x

Elnayef, B., Porta, C., Suarez-Lopez Del Amo, F., Mordini, L., Gargallo-Albiol, J., & Hernandez-Alfaro, F. (2018). The fate of lateral ridge augmentation: A systematic review and meta-analysis. *The International Journal of Oral & Maxillofacial Implants*, *33*(3), 622–635. https://doi.org/10.11607/jomi.6290

Friedmann, A., Strietzel, F. P., Maretzki, B., Pitaru, S., & Bernimoulin, J. P. (2002). Histological assessment of augmented jaw bone utilizing a new collagen barrier membrane compared to a standard barrier membrane to protect a granular bone substitute material. *Clinical Oral Implants Research*, *13*(6), 587–594.

Gordh, M., Alberius, P., Lindberg, L., & Johnell, O. (1997). Bone graft incorporation after cortical perforations of the host bed. *Otolaryngology and Head and Neck Surgery*, *117*(6), 664–670. https://doi.org/10.1016/s0194-5998(97)70050-0

Hämmerle, C. H., Jung, R. E., Yaman, D., & Lang, N. P. (2008). Ridge augmentation by applying bioresorbable membranes and deproteinized bovine bone mineral: A report of twelve consecutive cases. *Clinical Oral Implants Research*, *19*, 19–25.

Hellem, S., Astrand, P., Stenstrom, B., Engquist, B., Bengtsson, M., & Dahlgren, S. (2003). Implant treatment in combination with lateral augmentation of the alveolar process: A 3-year prospective study. *Clinical Implant Dentistry and Related Research*, *5*(4), 233–240.

Jensen, S. S., Broggini, N., Hjorting-Hansen, E., Schenk, R., & Buser, D. (2006). Bone healing and graft resorption of autograft, anorganic bovine bone and beta-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. *Clinical Oral Implants Research*, *17*(3), 237–243. https://doi.org/10.1111/j.1600-0501.2005.01257.x

Meloni, S. M., Jovanovic, S. A., Urban, I., Canullo, L., Pisano, M., & Tallarico, M. (2017). Horizontal ridge augmentation using GBR with a native collagen membrane and 1:1 ratio of Particulated xenograft and autologous bone: A 1-year prospective clinical study. *Clinical Implant Dentistry and Related Research*, *19*(1), 38–45. https://doi.org/10.1111/cid.12429

Mendoza-Azpur, G., de la Fuente, A., Chavez, E., Valdivia, E., & Khouly, I. (2019). Horizontal ridge augmentation with guided bone regeneration using particulate xenogenic bone substitutes with or without autogenous block grafts: A randomized controlled trial. *Clinical Implant Dentistry and Related Research*, *21*(4), 521–530. https://doi.org/10.1111/cid.12740

Mir-Mari, J., Benic, G. I., Valmaseda-Castellon, E., Hammerle, C. H. F., & Jung, R. E. (2017). Influence of wound closure on the volume stability of particulate and non-particulate GBR materials: An in vitro cone-beam computed tomographic examination. Part II. *Clin Oral Implants Res*, *28*(6), 631–639. https://doi.org/10.1111/clr.12845

Mir-Mari, J., Wui, H., Jung, R. E., Hammerle, C. H., & Benic, G. I. (2016). Influence of blinded wound closure on the volume stability of different GBR materials: An in vitro cone-beam computed tomographic examination. *Clinical Oral Implants Research*, *27*(2), 258–265. https://doi.org/10.1111/clr.12590

Monje, A., Chappuis, V., Monje, F., Muñoz, F., Wang, H. L., Urban, I. A., & Buser, D. (2019). The critical peri-implant buccal bone wall thickness revisited: An experimental study in the beagle dog. *The International Journal of Oral & Maxillofacial Implants*, *34*(6), 1328–1336. https://doi.org/10.11607/jomi.7657

Mordenfeld, A., Aludden, H., & Starch-Jensen, T. (2017). Lateral ridge augmentation with two different ratios of deproteinized bovine bone and autogenous bone: A 2-year follow-up of a randomized and controlled trial. *Clinical Implant Dentistry and Related Research*, *19*(5), 884–894. https://doi.org/10.1111/cid.12512

Mordenfeld, A., Hallman, M., Johansson, C. B., & Albrektsson, T. (2010). Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. *Clinical Oral Implants Research*, *21*(9), 961–970. https://doi.org/10.1111/j.1600-0501.2010.01939.x

Mordenfeld, A., Johansson, C. B., Albrektsson, T., & Hallman, M. (2014). A randomized and controlled clinical trial of two different compositions of deproteinized bovine bone and autogenous bone used for lateral ridge augmentation. *Clinical Oral Implants Research*, *25*(3), 310–320. https://doi.org/10.1111/clr.12143

Nyström, E., Nilson, H., Gunne, J., & Lundgren, S. (2009). A 9-14 year follow-up of onlay bone grafting in the atrophic maxilla. *International Journal of Oral and Maxillofacial Surgery*, *38*(2), 111–116. https://doi.org/10.1016/j.ijom.2008.10.008

Ortak, T., Ozdemir, R., Uysal, A., Ulusoy, M. G., Sungur, N., Sahin, B., Koçer, U., & Sensöz, O. (2005). Osteogenic capacities of periost grafts, periost flaps and prefabricated periosteal flaps: Experimental study. *The Journal of Craniofacial Surgery*, *16*(4), 594–600. https://doi.org/10.1097/01.scs.0000168773.71356.62

Pikdoken, L., Gurbuzer, B., Kucukodaci, Z., Urhan, M., Baris, E., & Tezulas, E. (2011). Scintigraphic, histologic, and histomorphometric analyses of bovine bone mineral and autogenous bone mixture in sinus floor augmentation: A randomized controlled trial—results after 4 months of healing. *Journal of Oral and Maxillofacial Surgery*, *69*(1), 160–169. https://doi.org/10.1016/j.joms.2010.07.036

R Development Core Team. (2008). *R: A language and Environment for Statistical Computing*. R Foundation for Statistical Computing.

Sbordone, C., Toti, P., Guidetti, F., Califano, L., Santoro, A., & Sbordone, L. (2012). Volume changes of iliac crest autogenous bone grafts after vertical and horizontal alveolar ridge augmentation of atrophic maxillas and mandibles: A 6-year computerized tomographic follow-up. *Journal of Oral and Maxillofacial Surgery*, *70*(11), 2559–2565. https://doi.org/10.1016/j.joms.2012.07.040

Song, Y. W., Jung, H. J., An, Y. Z., Jung, U. W., & Lee, J. S. (2021). Addition of autogenous bone chips to deproteinized bovine bone mineral does not have additional benefit in lateral ridge augmentation-a preclinical in vivo experimental study. *Clinical Oral Implants Research*, *32*(9), 1105–1114. https://doi.org/10.1111/clr.13805

Stavropoulos, F., Dahlin, C., Ruskin, J. D., & Johansson, C. (2004). A comparative study of barrier membranes as graft protectors in the treatment of localized bone defects. An experimental study in a canine model. *Clinical Oral Implants Research*, *15*(4), 435–442. https://doi.org/10.1111/j.1600-0501.2004.01029.x

Urban, I. A., Nagursky, H., & Lozada, J. L. (2011). Horizontal ridge augmentation with a resorbable membrane and particulated autogenous bone with or without anorganic bovine bone-derived mineral: A prospective case series in 22 patients. *The International Journal of Oral & Maxillofacial Implants*, *26*(2), 404–414.

Urban, I. A., Nagursky, H., Lozada, J. L., & Nagy, K. (2013). Horizontal ridge augmentation with a collagen membrane and a combination of particulated autogenous bone and anorganic bovine bone-derived mineral: A prospective case series in 25 patients. *The International Journal of Periodontics & Restorative Dentistry*, *33*(3), 299–307. https://doi.org/10.11607/prd.1407

Yushkevich, P. A., Piven, J., Hazlett, H. C., Smith, R. G., Ho, S., Gee, J. C., & Gerig, G. (2006). User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. *NeuroImage*, *31*(3), 1116–1128. https://doi.org/10.1016/j.neuroimage.2006.01.015

How to cite this article: Aludden, H., Starch-Jensen, T., Dahlin, C., Sdik, J., Cederlund, A., & Mordenfeld, A. (2024). Histological and radiological outcome after horizontal guided bone regeneration with bovine bone mineral alone or in combination with bone in edentulous atrophic maxilla: A randomized controlled trial. *Clinical Oral Implants Research*, *35*, 396–406. https://doi.org/10.1111/clr.14235