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

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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

Running title: Aludden et al. Radiographic changes after lateral augmentation

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Author contribution:
HA: Conceived the ideas, performed surgery, collected data, analyzed the data, led the writing
AM, CD: Conceived the ideas, analyzed the data, major contributions to the manuscript
AC, RSN: Conceived the ideas for collecting and analyzing data, collected the data, analyzed the data. Major contribution to the technical radiographical part of the manuscript
PVP: Convinced the ideas for and acquired the CT scans, collected data
BS: Performed the statistical analyses, major contribution to the result part of the manuscript
TSJ: Conceived the ideas, performed surgery, major contribution to the manuscript

Abstract
Objective: Estimate changes in augmentation height and volume after lateral guided bone regeneration (GBR) augmentation with different ratios of deproteinized bovine bone mineral (DBBM) and particulate autogenous bone (PAB) and autogenous bone block (ABB), at different time points.

Material and methods: Twenty-four minipigs were randomly allocated into three heling periods. Lateral augmentation in 96 sites with standardized quantity of graft material was performed with different ratios of DBBM and PAB (50:50, 75:25 and 100:0) and ABB in combination with DBBM, covered by a collagen membrane. Changes in augmentation height and volume were assessed on CT volumes acquired 10, 20, and 30 weeks after surgery.

Results: Reduction in bone augmentation height was: 50:50 - 1.7 mm (-33.1%), 75:25 - 1.8 mm (-37.8%), 100:0 - 1.7 mm (-35.8%), and ABB - 0.2 mm (-3.7%), after 30 weeks. The augmentation height was significantly better preserved with ABB compared to 50:50, 75:25, and 100:0, while no significant difference was present among particulate grafts. No significant difference in volumetric
reduction was found among 50:50, 75:25, 100:0 and ABB after 30 weeks, while 100:0 presented significant less reduction compared to 50:50, 75:25 and ABB after 10 and 20 weeks.

Conclusions: Augmentation height following GBR was better preserved with ABB covered with DBBM. Addition of PAB to DBBM did not affect the changes in height of the graft. The volumetric stability seems to be comparable for ABB covered by DBBM and all particulate grafts after 30 weeks. However, DBBM alone revealed significant less volume reduction in the early healing phase.

INTRODUCTION
Horizontal reconstruction of alveolar deficiencies prior to implant placement with autogenous bone block (ABB) is associated with significant resorption of the augmented area (Antoun, Sitbon, Martinez, Missika, 2001; Cordaro, Amade, Cordaro, 2002; Dasmah, Thor, Ekestubbe, Sennerby, Rasmusson, 2012; Gulinelli et al., 2017; Widmark et al., 1998). Hence, covering the ABB with a non-resorbable bone substitute in combination with a resorbable collagen membrane have been recommended to reduce the resorption rate during the remodelling process (Chappuis, Cavusoglu, Buser, von Arx, 2017; Meijndert et al., 2017; von Arx, Buser, 2006). However, harvesting of an ABB from intra- or extra oral donor sites is associated with risk of donor site morbidity, limited quantities of graft material and prolonged treatment time (Cordaro, Torsello, Morcavallo, di Torresanto, 2011; Cricchio, Lundgren, 2003; Pereira et al., 2015). Horizontal reconstruction of alveolar deficiencies using guided bone regeneration (GBR) with non-resorbable bone substitutes alone or in combination with particulate autogenous bone (PAB) have therefore been used increasingly to simplify the surgical procedure and reduce resorption of the graft material (Block, Ducote, Mercante, 2012; Chappuis et al.; Chen, Chang, Leung, Lai, Kao, 2007; Friedmann, Strietzel, Maretzki, Pitaru, Bernimoulin, 2002; Hellem et al., 2003; Mayfield, Skoglund, Hising, Lang, Attstrom, 2001; Meloni et al., 2017; Mendoza-Azpur, de la Fuente, Chavez, Valdivia, Khouly, 2019; Mordenfeld, Aludden, Starch-Jensen, 2017; Mordenfeld, Johansson, Albrektsson, Hallman, 2014; Urban, Nagursky, Lozada, 2011; Urban, Nagursky, Lozada, Nagy, 2013)

Sufficient bone regeneration, high survival rates of implants and suprastructures, limited peri-implant marginal bone loss, and low frequency of complications have been reported after horizontal reconstruction of alveolar deficiencies with different compositions of deproteinized
bovine bone mineral (DBBM) and PAB, as documented in systematic reviews (Al-Nawas, Schiegnitz, 2014; Aludden, Mordenfeld, Hallman, Dahlin, Jensen, 2017; Elnayef et al., 2018).

There have been inconsistent conclusions regarding the dimensional stability of different graft compositions. A newly published randomized controlled trial demonstrated no significant difference in augmentation height changes after horizontal reconstruction of alveolar deficiencies with DBBM alone compared with ABB covered by DBBM and a collagen membrane after 18 months (Mendoza-Azhur et al.). However, a previous systematic review concluded that ABB maintained the augmentation height better compared with particulate grafts (Troeltzsch et al., 2016), which has been confirmed in a later systematic review that also concluded that DBBM alone revealed reduced resorption of the augmented region compared with different ratios of DBBM and PAB (Elnayef et al., 2018).

Previous studies assessing augmentation height and volumetric changes of the graft material after horizontal reconstruction of alveolar deficiencies with either ABB or different compositions of particulate graft material have used different assessments methods, including clinical measurements, different protocols of the radiographic measurements, and different observation periods. The changes in augmentation height and volume of the graft material during the remodeling process seems to be influenced by the composition of the graft material and the healing period. However, to our knowledge, these parameters have never been systematically assessed following horizontal reconstruction of the alveolar ridge with different compositions of particulate graft material compared with ABB, after different healing periods.

Implant placement after lateral GBR procedures with ABB is usually performed after 5-6 months (Chappuis et al.; Gulinelli et al., 2017; Maiorana, Beretta, Salina, Santoro, 2005; von Arx, Buser), while the healing period typically is prolonged for particulate grafts with DBBM (Hammerle, Jung, Yaman, Lang, 2008; Meloni et al., 2017; Mendoza-Azhur et al.; Mordenfeld et al; Urban et al.2011; Urban et al., 2013).

The present study aimed to assess two healing periods to simulate healing periods, frequently used for lateral GBR procedures in humans. The mineralization and incorporation of the graft material, the quantity of the material and placement of the evaluated material were supposed to be comparable to the clinical situation for horizontal ridge augmentation. A shorter healing period was chosen to assess the changes in height and volume of the augmented area in the early healing phase.
Hence, the objective of the present study was to estimate the changes in augmentation height and volume of the graft material after lateral GBR procedures of the mandible with different ratios of DBBM and PAB, and ABB covered by DBBM and a resorbable membrane, at different time points using two- and three-dimensional CT-based measurements. The histological results are presented in a separate paper (Aludden, Mordenfeld, Dahlin, Starch-Jensen, 2020).

MATERIAL AND METHOD
Study design including drug administration and anesthesia have previously been described in detail (Aludden et al. 2020) and is shortly summarized below.

Ethical considerations
License was obtained from The Danish Experimental Animal Inspectorate, The Danish Veterinary and Food Administration, Ministry of Environment and Food of Denmark, Copenhagen, Denmark (Approval no. 2016-15-0201-00822). Study design and experimental procedures was conducted in accordance with ARRIVE guidelines for animal studies and directive 2010/63/EU.

Animals
Twenty-four minipigs were randomly allocated into three groups of eight animals for three different healing periods. A computer-generated randomization allocated numbers from one to 24 to a defined treatment sequence as well as if augmentation would be performed in the right or left side of the mandible. Prior to surgery, a number between one and 24 was drawn from a sealed envelope allocating the animal to the specific treatment. The augmentation procedure was performed in either the right or the left side of the mandible according to the allocated randomized number of each animal and the bone block was harvested from the opposite side to not interfere with the augmentation procedure. Lateral augmentation of the mandible was performed in all animals with four different ratios of DBBM and autogenous bone. Recipient site number one was positioned most posteriorly on the lateral surface of the mandible and number four was positioned most anteriorly. All animals had augmentation at four recipient sites according to a randomized treatment sequence. A total number of 96 sites were augmented (Table 1).
The animals used in the present study were part of a separate study as well, assessing histologic and histomorphometric changes over time.

**Surgical procedure**

*Harvesting of a mandibular bone block*

The lateral and inferior mandibular border was exposed through a submandibular skin incision. A 20 x 10 mm cortical bone block involving the lateral and inferior cortex was harvested with a fissure bur during continuous cooling with sterile saline solution.

*Lateral augmentation of the mandible*

*Preparation*

The lateral and inferior mandibular border was exposed through a submandibular skin incision in the contralateral side of the mandible. The lateral surface of the mandible was divided into four recipient sites. An osteosynthesis screw (Ø2.0 mm x 9 mm, Stryker Corporation, USA) was inserted at the inferior border of the mandible, corresponding to the midline of each augmented area. The screw was used as a reference landmark for orientation of the augmented area during the radiographic assessment.

*Different ratios of DBBM and bone for GBR*

Small size (0.25-1 mm) DBBM particles (Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland) was mixed with PAB by weight in the following ratios: 50:50) 50% DBBM and 50% PAB, 75:25) 75% DBBM and 25% PAB, and 100:0) 100% DBBM, ABB) Autogenous bone block covered by a composition of DBBM and PAB . A specially fabricated stainless-steel frame (10 x 10 x 5 mm) was used to ensure a standardized quantity of the particulate graft material at each recipient site (Figure 1). The augmented regions were covered with a resorbable, non-crosslinked collagen barrier membrane (Bio-Gide, Geistlich Pharma, Wolhusen, Switzerland) and fixed with four titanium pins (Dentsply Frios, Astra Tech, Molndal, Sweden).

The ABB was processed to a size of approximately 10 x 10 x 4 mm and applied passively to the lateral surface of the mandible. The ABB was fixed with an osteosynthesis screw and covered by a 1 mm layer of 50% DBBM and 50% PAB, estimated by weight. Finally, the augmented area was covered by a fixed resorbable barrier membrane as described above. Illustration of the position of the augmented sites on the lateral surface of the mandible is presented in figure 2.
Euthanasia and perfusion
Groups of eight minipigs were euthanized after 10, 20, and 30 weeks, respectively. The animals were deeply anaesthetized. The left and right common carotid arteries were exposed, cannulated with a catheter (Avanti, Cordia Cashel, Ireland) and perfused with 1000 ml neutral-buffered Ringer solution (2500ml/min) followed by 1000 ml neutral-buffered formaldehyde solution (2500ml/min).

Computed tomography
Computed tomography (CT) volumes (Discovery CT750 HD, General Electric Company, United States) were acquired with 0.625 mm section thickness and 0.312 mm distance between the sections. The animals were place in a supine position with a horizontal occlusal plane. Two sets of CT volumes were acquired, i.e. immediately after surgery, and after euthanasia. To provide blinding of the radiographic evaluation, the CT volumes were coded.

Changes in height of the augmented area
The dimensional (linear) changes of the augmented regions were measured by two-dimensional linear measurements on axial CT images acquired postoperatively and at euthanasia in Romexis software (version 5.3, Planmeca Oy, Finland) by an experienced specialist in oral radiology. A straight line was demarcated through each augmented region corresponding to the middle of the reference screw at the inferior mandibular border. Two parallel lines were drawn anteriorly and posteriorly at a distance of two millimeters from the first line, respectively (Figure 3). The vertical distance from the position screw to the center of the transplant was measured to find the correct position for the measurements in the corresponding volume at euthanasia. The initial width of the mandible was measured in millimeters from the outer lingual cortical surface to the outer facial cortical surface of the mandible at three points, corresponding to the three parallel lines. The width of the mandible including the height of the augmented region was measured in millimeters from the outer lingual cortical surface of the mandible to most lateral part of the augmented region at three points, corresponding to the three parallel lines. The height of each augmented region was estimated by subtracting the initial width of the mandible from the width of the mandible including the augmented height. A mean value was calculated for each of the augmented regions corresponding to the three lines on axial CT images obtained immediately postoperative and after
euthanasia. However, the border between the augmented region and the outer facial cortical surface of the mandible was difficult to distinguish on certain CT images at euthanasia. Therefore, the initial width of the mandible, which was measured on postoperative CT images was subtracted from the width of the mandible including the augmented region on CT images at euthanasia.

**Volumetric changes**

The volumetric changes of the augmented regions were measured by three-dimensional measurements on coronal CT-scan images obtained postoperatively and at euthanasia. The two sets of CT-scans were uniform orientated according to the lines using dedicated software (OnDemand 3D, CyberMed, Seul, South Korea). Initially, a straight line between the centers of the two most posterior membrane fixation pins was defined (Figure 4a). Perpendicular to the posterior line, a straight line in lateral-medial direction corresponding to the middle of the inferior fixation pin was demarcated (Figure 4b). The posterior line, which was located posteriorly for the first augmented region, served as a starting point for selection of CT-scan images. Coronal CT images from the starting point until the anterior membrane fixation pins of the fourth augmented region was selected with a distance of 2 mm between the CT sections, thereby ensuring an equal mutual distance between the 5 to 9 selected images of each augmented region. Selected images on the postoperative CT were assigned a number, which corresponded to identical CT sections obtained at euthanasia. The augmented region on the selected postoperative and euthanasia CT images were outlined with Image J software (National Institutes of Health, USA) (Figure 5a, b). The augmented volume (mm$^3$) on the selected CT images were counted in Image J software (National Institutes of Health, USA). The volume of each selected section was multiplied by 2, due to the 2 mm distance between sections, before the total volume for each of the augmented region was estimated by adding the counted volume value of each selected sections (i.e. Cavalieri’s principle). The volumetric changes of the augmented regions in mm$^3$ were finally calculated by subtraction the estimated volume of the augmented region on CT obtained at euthanasia from the estimate volume on postoperative CT volume.

**Statistical analyses**

A sample size calculation was conducted using a statistical software program (Stata 16.1, Stata Corp P, TX, USA). A sample size calculation based on an alpha significance level of 0.05 to achieve 80% power to detect a clinically meaningful difference of 8% in volume reduction (SD:
5%) between 2 of the investigated groups at one time point. The sample size calculation suggested that 8 animals in each of the 4 investigated groups were enough to detect a difference of 8% why 24 animals was regarded to be an adequate number for the study.

Data management and statistical analyses including the calculation of descriptive statistics were conducted using the statistical software R version 2.8.0. (R Development Core Team 2008). The primarily outcomes were changes in augmented height and volume. Data was analyzed in a mixed model for normally distributed data with percentage of changes in height and volume of the graft material as dependent variables. Ratio and position of the graft material on the lateral surface of the mandible was selected as fixed factors, while animal was used as random factor. The mixed models were fitted. Pairwise testing of differences in changes in height and volume of the graft material between each ratio was conducted. P-values and confidence intervals were adjusted for multiple testing within time points and ratio using Tukey’s method. Results were summarized as mean values with standard deviation and 95% confidence interval (CI). A statistically significant difference was considered at P < 0.05.

RESULTS

Uneventful healing was seen in all animals. One animal died before surgery due to infection with coccidian protozoa. The animal was substituted and eight minipigs were included in each group. CT-scans were acquired satisfactorily immediately after surgery and at euthanasia in all animals. Following the completion of the radiological analysis, the specimens were collected for analysis in a separate study.

The results are presented for 50:50) 50% DBBM and 50% PAB, 75:25) 75% DBBM and 25% PAB, 100:0) 100% DBBM and ABB.

Changes in height of the augmented area

The mean height of the augmented regions immediately after surgery (baseline) were compared with the height at 10 weeks, 20 weeks, and 30 weeks (Figure 6). The gained height was 3.1 mm (± 2.6) for 50:50, 3.0 mm (± 2.3) for 75:25, 2.9 mm (±1.0) for 100:0 and 4.6 mm (±1.4) for ABB after 30 weeks.
All particulate grafts used during GBR presented a significant height reduction from baseline compared with measurements at 10 weeks, 20 weeks and 30 weeks, while the ABB in combination with DBBM presented no significant reduction in height from baseline to 10, 20 and 30 weeks (Table 2). ABB presented significantly less height reduction compared to all the particulate grafts after 10 weeks (p=0.00), 20 weeks (p<0.01), and 30 weeks (p<0.05) (Table 3). There was no significant difference in height reduction between the particulate grafts at 10 weeks and 30 weeks but after 20 weeks, 50:50 presented significantly less reduction compared to 75:25.

Volumetric changes
The mean volume of the augmented regions immediately after surgery (baseline) were compared with the volume at euthanasia at 10 weeks, 20 weeks, and 30 weeks, respectively (Figure 7).

A significant reduction in volume was presented for 50:50 (p=0.00) and ABB (p=0.02) from baseline to 20 weeks (Table 4). There was no significant difference between any of the graft materials after 30 weeks (Table 5). However, 100:0 revealed significantly less volume reduction compared to ABB (p=0.01) and 75:25 (p=0.01) after 10 weeks and compared to ABB, 50:50 and 75:25 at 20 weeks (p=0.00). A significantly reduced volume was presented for 50:50 compared with 75:25 (p=0.04) at 10 weeks, whereas 75:25 revealed a significantly reduced volumetric reduction compared with 50:50 (p=0.02) at 20 weeks.

DISCUSSION
In the present study, two-dimensional (linear) measurements on CT volumes demonstrated significantly less height reduction with ABB covered by DBBM and a resorbable membrane compared with different ratios of DBBM and PAB. However, the addition of PAB to DBBM did not seem to affect the resorption rate.

Horizontal reconstruction of alveolar deficiencies prior to implant placement requires a graft material that can withstand biodegradation and maintain the long-term dimension of the augmented alveolar ridge. DBBM is biocompatible and possesses osteoconductive properties when used as a material in conjunction with GBR procedures and as a scaffold for ingrowth of new bone. The inorganic bone matrix of DBBM appears to have a microscopic structure similar to human cancellous bone and a long-term study have indicated no or limited resorption of the DBBM (Mordenfeld, Hallman, Johansson, Albrektsson, 2010). DBBM alone or in combination...
with PAB seems to be a suitable graft material for lateral GBR reconstruction of alveolar deficiencies prior to implant placement.

Lateral augmentation with ABB can result in an extensive resorption of the bone block as a consequence of remodeling during graft healing and incorporation into the osseous recipient site and different methods have been applied to reduce the resorption. To cover the ABB with a non-resorbable ePTFE membrane according to the guided bone regeneration (GBR) technique, have previously been documented to minimize the resorption of the ABB with satisfactory results (Buser, Dula, Hirt, Schenk, 1996). To simplify the technique, resorbable collagen membranes have been utilized. Due to the short duration of barrier function, a non-resorbable bone substitute have been applied on the surface of the ABB to protect the bone block from surface resorption (Cordaro et al., 2011; von Arx, Buser).

This technique, combining the ABB with a graft protective material and the use of the GBR principle with a resorbable membrane has revealed a resorption rate of 7% after 10 years (Chappuis et al.), compared to 9.3% with coverage only by DBBM (Maigorana et al., 2005) or no coverage of the ABB (18-60%) (Antoun et al.; Cordaro et al.; Dasmah et al.; Maigorana et al.; Sbordone et al., 2009).

An experimental study in sheep presented a reduced reduction of the block graft when applying a resorbable collagen membrane alone or in combination with DBBM compared to ABB alone. However, it was concluded that maintenance of the graft was dependent on the stability of the membrane during healing (Adeyemo et al., 2008).

The resorption rate of particulate grafts applying the GBR technique with resorbable collagen membranes varies in the literature. Fixation of the membrane by pins or tacks have presented a superior gain in height of the alveolar process up to 5.6 mm (Meloni et al., 2017; Mendoza-Azpuru et al.; Urban et al., 2011, 2013) compared to no fixation of the graft where up to 3.5 mm was presented (Mordenfeld et al.), which confirms the previous statement of the importance of membrane stability (Adeyemo et al., 2008). Comparing height changes among studies can be challenging since it probably is of importance how the radiographic measurements are performed as discusses by Mendoza-Azpuru et al. (2019) as well as the site of the measurement (vertically) since it has been documented that the graft presents an increased resorption close to the top of the alveolar process compared to the more apical part of the graft (Mir-Mari, Wui, Jung, Hammerle, Benic, 2016; Mordenfeld et al.).
This discrepancy in the results for two-dimensional measurements in the present study, could be caused by a higher resistance for pressure for the ABB compared to the particulate grafts since the augmentation of the mandible was performed outside the skeletal envelope and pressure against the augmented region was difficult to avoid due to the postoperative behavior of the animals, which could cause displacement of the graft material. A previous systematic review has presented a difference of 1 mm in gained width, favorable for the ABB compared to particulate grafts (Troeltzsch et al., 2016). The difference in the present study exceeds 1 mm and the resorption rate was 33-38% for the particulate grafts compared to only 1.3% for the ABB and an explanation could be the displacement of the particulate graft. Though, it can be discussed if this resorption rate has a clinical impact since a randomized clinical study with a split mouth protocol in edentulous patients presented a resorption rate of 37% for a 60:40 mixture and 47% for at 90:10 mixture and were all the planned implants except for one could be installed in 13 patients (Mordenfeld et al.). An increased width was observed after 30 weeks in some materials. This could might be explained by a periosteal driven bone formation which previously have been observed in conjunction with augmentation procedures (Mordenfeld, Hallman, Lindskog, 2011).

Regarding the three-dimensional measurements on CT-volumes, the present study presented comparable volumetric changes with different ratios of DBBM and PAB and for ABB in combination with DBBM after 30 weeks. However, the results from the volumetric measurements at 30 weeks should be regarded with caution, since the volume of the augmented regions were generally increased at 30 weeks compared with measurements at 20 weeks. This could be explained by a GBR effect from tenting of the periosteum since the increased bone volume primarily was observed inferiorly, superiorly, anteriorly and posteriorly to the grafted region. Hence, it did not affect the height measurements. The reduction at 20 weeks was 18-37% for different compositions of DBBM and PAB, including the ABB covered by DBBM. The graft consisting of 100% DBBM maintained the volume significantly better compared to all other grafts. Since DBBM is a non-resorbable bone substitute, this could explain the superior volumetric stability, especially since the grafts presenting the highest reduction were the graft with the highest amount of autogenous bone (i.e. the bone block and the 50:50 ratio).

However, a histological trial in the same study animals presented no significant difference on bone formation among the particulate grafts (Aludden et al. 2020).
Studies presenting volumetric changes assessed by three-dimensional measurements after horizontal ridge augmentation are sparse. Three-dimensional measurements on cone beam CT volumes for two different compositions of DBBM and PAB (90:10 and 60:40) revealed a volume reduction of 55.3% and 53.8% respectively after 8 months. There was no significant difference in volume reduction between the two compositions (Mordenfeld et al.). A retrospective study evaluating the volume reduction of an ABB from the iliac crest compared to a particulate graft demonstrated a reduced volume reduction for the particulate graft compared to the ABB (Gultekin, Cansiz, Borahan, 2017). An experimental study evaluating different compositions of DBBM and PAB placed in cylinders in rabbit calvaria used clinical measurements with a probe to calculate the volume of the different grafts, after 12 weeks (Kim et al., 2020) and demonstrated a reduced reduction in volume for DBBM alone compared to PAB alone or compared to different compositions of DBBM and PAB. A preclinical study in minipigs assessing different ratios of DBBM and PAB in conjunction with maxillary sinus floor augmentation demonstrated that the volumetric changes of the graft material was significantly influenced by the ratio of DBBM and PAB with minimal resorption of the graft material when DBBM was used alone after 12 weeks (Jensen et al., 2012). However, volumetric changes in a closed compartment like a metallic cylinder or the maxillary sinus is difficult to compare with the lateral situation since no pressure will be applied and therefore displace the graft and the possible tenting effect of the periosteum is not present. Consequently, there is no conclusive results regarding the volumetric stability after horizontal ridge augmentation when comparing autogenous bone or grafts consisting of different compositions of DBBM and PAB.

The present study indicates a favorable height stability of the augmented region after lateral GBR procedures with the combination of ABB and DBBM compared to particulate grafts. However, the volumetric stability seems to be superior for 100% DBBM compared to particulate graft with addition of PAB as well as the ABB covered by DBBM. Though, this study does not report the influence of the presented changes in height and volume on implant survival.

Experimental studies present limitations and results are not definitely applicable on humans due to differences in anatomy, metabolism and physiology. However, the bone remodeling rate, bone mineral density and healing potential in minipigs seems to be comparable to humans (Reinholz, Lu, Saris, Yaszemsksi, & O'Driscoll, 2004), and previous studies have used minipigs for assessment of bone regeneration after augmentation of the alveolar ridge with different compositions of DBBM and PAB.
biomaterials (Jensen, Broggini, Hjorting-Hansen, Schenk, Buser, 2006; Jensen et al., 2013; Jensen et al., 2012).

However, the results of the present preclinical trial should be regarded with caution since the augmentation procedure was performed outside the skeletal envelope and in a location on the lateral aspect of the mandible, where a periosteal driven bone formation cannot be excluded. However, this is equally distributed between the respective groups.

Further randomized clinical trials assessing, clinical, radiological and histomorphometric outcomes after horizontal reconstruction of alveolar deficiencies with different ratios of DBBM and PAB are needed before particulate graft materials can replace the ABB covered with DBBM to reduce harvesting of bone.

Conclusion

Within the limitations of the present study, significantly less reduction in height of the GBR augmented region was shown for ABB covered with DBBM and a resorbable collagen membrane, compared to different ratios of DBBM and PAB. DBBM alone revealed significant less volumetric reduction compared with different ratios of DBBM and PAB and the ABB covered by DBBM after 10 weeks and 20 weeks, respectively when used in combination with GBR. The addition of PAB to DBBM did not improve the height or volumetric stability.

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REFERENCES


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Figure Legends

*Figure 1.* Illustration of the graft material placed in a stainless-steel frame to ensure a standardized quantity of graft material.

*Figure 2.* Illustration of the recipient sites and the size of the grafted areas.

*Figure 3.* Illustration of the bone augmentation height measurements.

*Figure 4a.* Illustration of the first line drawn inferior-superior between the two most posteriorly placed fixation pins.

*Figure 4b.* Illustration of the line drawn in a lateral-medial direction along the direction of the superior fixation pin used for drawing the line in figure 3a.

*Figure 5a.* Illustration of outlining the augmented area in the postoperative CT volume.

*Figure 5b.* Illustration of outlining the augmented area in the CT volume from euthanasia.

*Figure 6.* Mean values and standard deviations of the augmented height at 10, 20, and 30 weeks, respectively.

*Figure 7.* Mean volumetric changes and standard deviation at 10, 20 and 30 weeks respectively.
**TABLE 1.** Randomization table

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Side</th>
<th>Healing period (weeks)</th>
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<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>R</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>L</td>
<td>10</td>
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<tr>
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<tr>
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<td>75:25</td>
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<td>100:0</td>
<td>ABB</td>
<td>L</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
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<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>R</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
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<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>L</td>
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</tr>
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<td>7</td>
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<td>ABB</td>
<td>75:25</td>
<td>R</td>
<td>10</td>
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<td>100:0</td>
<td>ABB</td>
<td>L</td>
<td>10</td>
</tr>
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<td>9</td>
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<td>50:50</td>
<td>100:0</td>
<td>R</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>L</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
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<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>R</td>
<td>20</td>
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<td>100:0</td>
<td>ABB</td>
<td>L</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>R</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>L</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>50:50</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>R</td>
<td>20</td>
</tr>
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<td>16</td>
<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>ABB</td>
<td>L</td>
<td>20</td>
</tr>
<tr>
<td>17</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>R</td>
<td>30</td>
</tr>
<tr>
<td>18</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>L</td>
<td>30</td>
</tr>
<tr>
<td>19</td>
<td>50:50</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>R</td>
<td>30</td>
</tr>
<tr>
<td>20</td>
<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>ABB</td>
<td>L</td>
<td>30</td>
</tr>
<tr>
<td>21</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>R</td>
<td>30</td>
</tr>
<tr>
<td>22</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>50:50</td>
<td>L</td>
<td>30</td>
</tr>
<tr>
<td>23</td>
<td>50:50</td>
<td>100:0</td>
<td>ABB</td>
<td>75:25</td>
<td>R</td>
<td>30</td>
</tr>
<tr>
<td>24</td>
<td>75:25</td>
<td>50:50</td>
<td>100:0</td>
<td>ABB</td>
<td>L</td>
<td>30</td>
</tr>
</tbody>
</table>

ABB: autogenous bone block; DBBM: deproteinized bovine bone mineral; L: augmentation left side of the mandible; PAB: particulate autogenous bone; R: augmentation right side of the mandible.  
50:50 corresponds to 50% DBBM & 50% PAB; 75:25 corresponds to 75% DBBM & 25% PAB; 100:0 corresponds to 100% DBBM.
<table>
<thead>
<tr>
<th>Graft</th>
<th>Baseline</th>
<th>10 weeks</th>
<th>20 weeks</th>
<th>30 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GW (SD)</td>
<td>GW (SD)</td>
<td>WC</td>
<td>CI</td>
</tr>
<tr>
<td>50:50</td>
<td>4.9 (±0.5)</td>
<td>2.8 (±1.3)</td>
<td>-2.1</td>
<td>-3.2; -1.0</td>
</tr>
<tr>
<td>75:25</td>
<td>5.2 (±0.9)</td>
<td>4.0 (±1.3)</td>
<td>-1.2</td>
<td>-2.3; -0.1</td>
</tr>
<tr>
<td>100:0</td>
<td>4.8 (±0.6)</td>
<td>3.5 (±1.4)</td>
<td>-1.3</td>
<td>-2.4; -0.2</td>
</tr>
<tr>
<td>ABB</td>
<td>4.4 (±0.8)</td>
<td>4.4 (±1.0)</td>
<td>0.0</td>
<td>0.8; 1.6</td>
</tr>
</tbody>
</table>

**TABLE 2.** Augmentation height changes in mm over time for the different graft materials

ABB: autogenous bone block; CI: confidence interval; DBBM: deproteinized bovine bone mineral; GW: gained width; PAB: particulate autogenous bone. 50:50 corresponds to 50% DBBM & 50% PAB; 75:25 corresponds to 75% DBBM & 25% PAB; 100:0 corresponds to 100% DBBM; SD: standard deviation; *

Statistically significant difference (p<0.05).
### TABLE 3. The contrast in height reduction between the different grafts at different time points

<table>
<thead>
<tr>
<th></th>
<th>10 weeks</th>
<th>20 weeks</th>
<th>30 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduction contrast</td>
<td>p value</td>
<td>Reduction contrast</td>
</tr>
<tr>
<td>50:50 vs 75:25</td>
<td>50:50 &gt; 75:25 (18.3%)</td>
<td>0.06</td>
<td>50:50 &gt; 75:25 (32.8%)</td>
</tr>
<tr>
<td>50:50 vs 100:0</td>
<td>50:50 &gt; 100:0 (14.5%)</td>
<td>0.13</td>
<td>50:50 &gt; 100:0 (5.8%)</td>
</tr>
<tr>
<td>50:50 vs ABB</td>
<td>50:50 &gt; ABB (49.3%)</td>
<td>0.00*</td>
<td>50:50 &gt; ABB (46.8%)</td>
</tr>
<tr>
<td>75:25 vs 100:0</td>
<td>100:0 &gt; 75:25 (3.7%)</td>
<td>0.70</td>
<td>100:0 &gt; 75:25 (27.0%)</td>
</tr>
<tr>
<td>75:25 vs ABB</td>
<td>75:25 &gt; ABB (31.0%)</td>
<td>0.00*</td>
<td>75:25 &gt; ABB (14.0%)</td>
</tr>
<tr>
<td>100:0 vs ABB</td>
<td>100:0 &gt; ABB (34.8%)</td>
<td>0.00*</td>
<td>100:0 &gt; ABB (41.0%)</td>
</tr>
</tbody>
</table>

ABB: autogenous bone block; DBBM: deproteinized bovine bone mineral; PAB: particulate autogenous bone. 50:50 corresponds to 50% DBBM & 50% PAB; 75:25 corresponds to 75% DBBM & 25% PAB; 100:0 corresponds to 100% DBBM; *: Statistically significant difference (p<0.05).
## TABLE 4.

Volumetric changes over time for the different grafts

<table>
<thead>
<tr>
<th>Graft composition</th>
<th>10 weeks</th>
<th>20 weeks</th>
<th>30 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume (SD)</td>
<td>VC (CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>50:50</td>
<td>608 mm³ (± 166)</td>
<td>0.6% (-22.0; 23.3)</td>
<td>0.96</td>
</tr>
<tr>
<td>75:25</td>
<td>488 mm³ (± 166)</td>
<td>-18.0% (-40.1; 4.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>100:0</td>
<td>718 mm³ (± 123)</td>
<td>7.8% (-15.0; 30.5)</td>
<td>0.50</td>
</tr>
<tr>
<td>ABB</td>
<td>416 mm³ (± 87)</td>
<td>-15.9% (-38.6; 6.9)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

ABB: autogenous bone block; CI: confidence interval; DBBM: deproteinized bovine bone mineral; PAB: particulate autogenous bone; VC: volumetric change. 50:50 corresponds to 50% DBBM & 50% PAB; 75:25 corresponds to 75% DBBM & 25% PAB; 100:0 corresponds to 100% DBBM; SD: standard deviation; *:Statistically significant difference (p<0.05).

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TABLE 5. The contrast in volume reduction between the different grafts at different time points

<table>
<thead>
<tr>
<th></th>
<th>10 weeks</th>
<th>20 weeks</th>
<th>30 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduction contrast</td>
<td>p value</td>
<td>Reduction contrast</td>
</tr>
<tr>
<td>50:50 vs 75:25</td>
<td>75:25 &gt; 50:50 (18.5%)</td>
<td>0.04*</td>
<td>50:50 &gt; 75:25 (19.0%)</td>
</tr>
<tr>
<td>50:50 vs 100:0</td>
<td>50:50 &gt; 100:0 (7.1%)</td>
<td>0.42</td>
<td>50:50 &gt; 100:0 (51.0%)</td>
</tr>
<tr>
<td></td>
<td>50:50 vs ABB</td>
<td>75:25 vs 100:0</td>
<td>75:25 vs ABB</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>----------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>50:50 vs ABB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABB &gt; 50:50</td>
<td>16.5%</td>
<td>0.00*</td>
<td>0.00*</td>
</tr>
<tr>
<td>0.06</td>
<td>25.7%</td>
<td>0.81</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>75:25 vs 100:0</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABB &gt; 75:25</td>
<td>2.1%</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>0.01*</td>
<td>23.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>75:25 vs ABB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABB &gt; 75:25</td>
<td>7.8%</td>
<td>0.33</td>
<td>0.00*</td>
</tr>
<tr>
<td>0.00*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>100:0 vs ABB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABB &gt; 100:0</td>
<td>39.7%</td>
<td>0.00*</td>
<td>0.74</td>
</tr>
</tbody>
</table>

50:50 corresponds to 50% DBBM & 50% PAB; 75:25 corresponds to 75% DBBM & 25% PAB; 100:0 corresponds to 100% DBBM; *: Statistically significant difference (p<0.05).