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

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Histological and histomorphometrical outcome after lateral GBR augmentation of the mandible with different ratios of deproteinized bovine bone mineral and autogenous bone. A preclinical in vivo study.

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Abstract

Objective: To test the hypotheses of no differences in (I) percentage of bone (POB), non-mineralized tissue (NMT) and deproteinized bovine bone mineral (DBBM), and (II) ingrowth of mineralized bone after lateral guided bone regeneration (GBR) augmentation of the mandible with different ratios of DBBM and particulate autogenous bone (PAB) at different time points.

Material and methods: Twenty-four minipigs were randomly allocated into three groups. Lateral augmentation in 96 sites (4 in each animal) was performed unilaterally with a standardized quantity of grafting material in each animal with different ratios of DBBM and PAB (50:50, 75:25, 100:0) and autogenous bone block in combination with DBBM and covered with a collagen membrane. The percentage of different tissues in the graft and ingrowth of mineralized bone was assessed by histomorphometric and histological analyses after 10, 20 and 30 weeks, respectively.

Results: The POB was 54% (50:50), 50% (75:25), and 48% (100:0) after 10 weeks, 60% (50:50), 61% (75:25), and 60% (100:0) after 20 weeks, 63% (50:50), 62% (75:25), and 62% (100:0) after 30 weeks. There was no significant difference between the groups at any time points. There was a significant increase in POB and a significant decrease in NMT for 75:25 and 100:0 from 10 to 30 weeks. All ratios demonstrated a non-complete ingrowth of mineralized bone into the graft after 10 weeks and complete mineralization after 30 weeks.

Conclusion: Within the limitations of the present study, it seems like addition of autogenous bone to DBBM for LRA did not affect the bone formation nor graft incorporation after 10 to 30 weeks of healing. However, a prolonged healing time seems to result in an increased POB for all ratios.

INTRODUCTION

Horizontal and vertical augmentation of the alveolar process prior to implant placement is frequently necessary when the dimension of the alveolar process is inadequate due to post-extraction defects, periodontal disease, traumatic tooth avulsion or long-term edentulism.

Lateral ridge augmentation (LRA) with an autogenous bone block (ABB) has been considered the preferred treatment modality for horizontal reconstruction of alveolar ridge deficiencies due to its osteoinductive and osteoconductive properties (Antoun, Sitbon, Martinez, Missika, 2001; Buser, Dula, Hirt, Schenk, 1996; Chappuis, Cavusoglu, Buser, von Arx, 2016; Cordaro, Torsello, Morcavallo, di Torresanto, 2011; Maiorana et al., 2011; Meijndert et al., 2017; Proussaefs, Lozada, 2003). However, the use of ABB is associated with risk of donor site morbidity and unpredictable resorption of the augmented area (Antoun, et al.; Cordaro, Amade, Cordaro, 2002; Cricchio, Lundgren, 2003; Dasmah, Thor, Ekestubbe, Sennerby, Rasmusson, 2012; Hallman, Thor, 2008). To simplify the surgical procedure and reduce the need for bone harvesting, guided bone regeneration (GBR) with various bone substitutes, alone or in combination with particulate autogenous bone (PAB) have been suggested for reconstruction of alveolar deficiencies (Block, Ducote, Mercante, 2012; Chappuis, et al.; Friedmann, Strietzel, Maretzki, Pitaru, Bernimoulin, 2002; Hammerle, Jung, Yaman, Lang, 2008; Hellem et al., 2003; Mayfield, Skoglund, Hising, Lang, Attstrom, 2001; Mordenfeld, Johansson, Albrektsson, Hallman, 2014; Rocchietta, Fontana, Simion, 2008).

One of the most documented bone substitutes for intraoral augmentation is deproteinized bovine bone mineral (DBBM). It possesses excellent osteoconductive properties and to add osteoinductive properties to the graft material, different ratios of PAB has been mixed with DBBM and assessed for lateral GBR augmentation, vertical augmentation and maxillary sinus floor augmentation (Block et al.; Friedmann et al.; Hallman, Lundgren, Sennerby, 2001; Hellem et al., 2003; John, Wenz, 2004; Meloni et al., 2017; Mendoza-Azpur, de la Fuente, Chavez, Valdivia, Khouly, 2019; Mordenfeld, Aludden, Starch-Jensen, 2017; Mordenfeld et al.; Simion, et al., 2007; Urban, Nagursky, Lozada, 2011; Urban, Nagursky, Lozada, Nagy, 2013). It has been suggested that the ratio of DBBM and PAB may influence the bone remodeling pattern (Galindo-Moreno et al., 2011) and that DBBM may interfere with bone formation in the early healing phase (Araujo, Linder, Lindhe, 2009; Araujo, Lindhe, 2011; Artzi et al., 2004; Jensen, Broggini, Hjorting-Hansen, Schenk, Buser, 2006; Stavropoulos, Kostopoulos, Mardas, Nyengaard, Karring, 2001). On the other hand, a randomized controlled trial, evaluating bone formation histologically and

scintigraphically after sinus floor augmentation, demonstrated no difference in bone formation between 100% DBBM and a mixture of DBBM and PAB (Pikdoken et al., 2011).

Studies on LRA, presenting histological assessment or comparison between different ratios of DBBM and PAB are sparse. A clinical randomized controlled trial with two different ratios of DBBM and PAB (90:10 and 60:40) demonstrated no significant difference in percentage of bone (POB) or ingrowth of mineralized bone into the graft between the two ratios. Clinically, no differences were experienced regarding graft healing, width of the alveolar crest or density of the graft during drilling for implants (Mordenfeld et al.). Two clinical studies evaluating LRA with 100% DBBM presented formation of new bone, comparable to grafts with addition of PAB and concluded that DBBM alone may be a suitable material for LRA in humans (Friedmann et al.; Zitzmann, Scharer, Marinello, Schupbach, Berglundh, 2001). However, the optimal composition of DBBM and PAB and its effect on the consolidation period is still unknown for lateral augmentation. Therefore, the objective of the present experimental study is to test the hypothesis of no difference in POB, percentage of NMT and percentage of DBBM as well as ingrowth of mineralized bone into the graft after lateral GBR augmentation of the mandible with different ratios of DBBM and PAB at different time points.

MATERIAL AND METHOD

Ethical considerations

A license to perform the study 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). The study design follows the ARRIVE guidelines for animal studies and the experiment was performed in accordance with directive 2010/63/EU.

Animals

The study included 24 adult, female Göttingen minipigs (Ellegaard Göttingen Minipigs A/S, Dalmose, Denmark) with a mean age of 18 months (range: 17-19 months) and a mean weight of 31 kg (range: 28-36.5 kg). During the study, the animals were fed daily with standard laboratory diet (Altromin 9023, Altromin International Gmbh, Lage, Germany) and water ad libitum.

The animals used in the present study were part of a separate study as well, assessing radiological changes over time.

Drug administration

Anesthesia

Anesthesia was induced by an intramuscular injection in the neck region with a mixture of zoleteil (0.125 ml/kg, Rompun, Bayer Health care AG, Leverkusen, Germany), ketamine (1.6 mg/kg, Ketaminol, Intervet International B.V, Boxmeer, the Netherlands), and butorfanol (0.3 mg/kg, Torbugesic, Fort Dodge Veterinaria S.A., Girona, Spain). For anesthesia, a standard straight 5.5 mm orotracheal tube with a cuff (Portex, Kent, UK) was placed and anesthesia was maintained by inhalation anesthesia with 1% sevoflurane (Forene, Abott Gmbh, Wiesbaden, Germany). The animals received a continuous intravenous infusion through an ear vein of a physiological saline slution containing propofol 10 mg/ml (4 mg/kg) and Fentanyl 50 mikrogram/ml (0.03 mikrogram/kg) during the surgical procedure.

Antibiotics

An intramuscular injection with Curamox® Prolongatum Vet, Amoxicillinum Trihydricum, 150 mg/ml (0.1 ml/kg, Meda AS, Allerød, Denmark) was given one hour before surgery. Peroral Imacillin 50 mg/ml (14 ml/kg, Meda AS, Allerød, Denmark) was given twice a day on the third and fourth day postoperatively.

Pain control

An intramuscular injection with Metacam®, 5 mg/ml (2 ml/25 kg, Boehringer Ingelheim 3 A/S) was given preoperatively. Metacam®, oral suspension for pigs, 15 mg/ml, (2.7 ml/100 kg, Boehringer Ingelheim Denmark A/S) was given peroral for five days postoperatively.

Surgical procedure

Twenty-four minipigs were randomly allocated into three groups of eight animals. A computer-generated randomization allocated each animal to a defined treatment sequence as well as if augmentation would be performed in the right or left side of the mandible. 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: A) 50% DBBM and 50% PAB, B) 75% DBBM and 25% PAB, C) 100% DBBM and D) ABB. The ABB was mainly used due to its part of another radiologic study. All animals had augmentation at four recipient sites according to a randomized treatment sequence. A total number of 96 sites were augmented.

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. A chisel was used to finish the osteotomy. The periosteum and skin were readapted and sutured in layers with Vicryl 3-0 and Nylon 3-0 (Ethicon, Norderstedt, Germany). Finally, the wound was covered by tissue glue (Leukosan Adhesive, BSN medical GmbH, Germany).

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 from where the bone block was harvested. The soft tissue was dissected to expose the lateral surface of the mandible. The mental foramen including the neurovascular bundle was identified and protected. The lateral surface of the mandible was divided into four recipient sites. Recipient site one was positioned 15 mm from the posterior border of the mandibular ramus with an inter-recipient site distance of 10 mm. The lateral cortex of the mandible at each recipient site was perforated with nine holes using a small round bur (Ø1.2 mm, Stryker Corporation, USA) under saline solution irrigation. Osteosynthesis screws (Ø2.0 mm x 9 mm, Stryker Corporation, USA) were placed close to the inferior border of the mandible, corresponding to the midline of each augmented area, as a reference landmark for orientation at the later histological preparation (Figure 1).

Bone block

The harvested bone block was cut in two pieces (10 x 10 x 4 mm). One block was prepared and applied passively to the lateral surface of the mandible and fixed with a titanium screw (Ø2.0 x 9

mm, Stryker Corporation, USA), primarily to be used for analysis in a separate study. The remaining bone block were particulate in a bone-mill (Roswitha Quétin Dentalprodukte, Germany) with 3 mm perforations to obtain bone particles with a size of 0.5-2 mm.

Different ratios of DBBM and PAB for GBR

Small size (0.25-1 mm) DBBM particles (Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland) was mixed with PAB by weight in three different ratios (50:50, 75:25 and 100:0). The graft material was soaked in autogenous blood from an ear vein prior to placement. A specially fabricated stainless-steel frame (10 x 10 x 5 mm) was used to ensure a standardized quantity of the graft material at each recipient site. The grafting material was packed layer by layer with a firm pressure until the stainless-steel frame was filled (Figure 2). After removal of the frame, all augmented areas were covered with a resorbable barrier membrane (Bio-Gide, Geistlich Pharma, Wolhusen, Switzerland) and fixed with four titanium pins (Dentsply Frios, Astra Tech, Molndal, Sweden) (Figure 3). The periosteum and skin were readapted and sutured in layers with Vicryl 3-0 and Nylon 3-0 (Ethicon, Norderstedt, Germany). Finally, the wound was covered by tissue glue (Leukosan Adhesive, BSN medical GmbH, Germany).

Euthanasia and perfusion

Groups of eight animals were euthanized after 10 weeks, 20 weeks and 30 weeks, respectively. The animals were deeply anaesthetized. The left and right common carotid arteries were exposed and dissected through a midline incision of the neck from the thyroid cartilage to just above the suprasternal notch. The carotid arteries were cannulated with a catheter (Avanti, Cordia Cashel, Ireland) and perfused with 1000 ml neutral-buffered Ringer solution (2500 ml/min) followed by 1000 ml neutral-buffered formaldehyde solution (2500 ml/min).

Histology

Specimens

Each specimen was immersed in 10% buffered formalin until the histological preparation was initiated. The specimens were rinsed in water followed by stepwise dehydration in a graded ethanol series, followed by embedding in plastic resin (LR White, London Resin Co.Ltd, UK). The embedded blocks were bisected corresponding to the reference screw inserted as a landmark inferior of each augmented area. Both bisected blocks of each specimen were used to prepare a 50

μm thick central ground section (EXAKT1 Apparatebau GmbH & Co, Norderstedt, Germany) (Donath, Breuner, 1982) stained with toluidine blue. All sections were coded and evaluated blindly by one author (HA). Qualitative and quantitative histology and histomorphometry were performed using light optical microscopy (Nikon Eclipse E600), following a calibration regarding different tissue types among the authors. All measurements were performed manually, directly on the computer screen connected to a microscope in Nikon NIS elements software. A four times magnification was used. In each specimen, a region of interest (ROI) was randomly selected. Following histological and histomorphometric measurements were made in all specimens:

1. POB, percentage of NMT and percentage of DBBM
2. Height of the augmented area (mm)
3. Ingrowth of mineralized bone into the augmented area (percentage of the total height)

The histomorphometric analyses did not discriminate between newly formed bone and autogenous bone from the transplant since this primarily is of interest when assessing the early mechanism in bone formation. This study is primary focusing on the total POB prior to implant placement.

To assess the ingrowth of mineralized bone a straight line was drawn from the host bone to the lateral border in the central part of the transplant. Two supplementary straight lines were drawn parallel to the first line but 1 mm anterior and posterior for it. A mean value (mm) for the three lines were calculated and acted as the value of the total height of the transplant.

Three new lines from the host bone to the lateral border of the mineralized part of the transplant were drawn. One in the central part of the transplant and two parallel lines 1 mm anterior and 1 mm posterior for the first line. A mean value (mm) were calculated and acted as the value of the height of mineralized bone. The height of mineralized bone was divided with the total height of the transplant and resulted in a value corresponding to the percentage of ingrowth of mineralized bone into to the transplant (Figure 4).

Statistical analysis

A sample size calculation was conducted based on a separate study on the same animals assessing the radiological outcome, using a statistical software program (Stata 16.1, Stata Corp P, TX, USA). A power calculation based on the radiological evaluation on same animals was conducted to define an adequate number of animals. 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 outcome was percentage of bone, percentage of NMT and percentage of DBBM. The data were analyzed in a mixed model for normally distributed data with percentage of bone, percentage of NMT and percentage of DBBM as dependent variable. Ratio, time points, interaction between time point, ratio and position as fixed factors, and animal as a random factor. The specified correlation structure allowed the error variance to be different at different time points. The mixed models were fitted using the function lme from the R package nmle. Pairwise testing of differences in percentage of bone, percentage of NMT and percentage of DBBM between time points within ratios and between ratios within time points were performed based on marginal means of the mixed models. 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 95% confidence interval. A statistically significant difference was considered at $p<0.05$.

RESULTS

One animal died before surgery due to infection with coccidian protozoa which was not related to the study. The animal was replaced, and eight animals were included in each group. Uneventful healing was seen in all animals. Histomorphometrical analyses were not possible in six specimens due to artefacts generated during histological processing. Hence, the histomorphometric analysis included 90 specimens as illustrated in Table 1.

General histologic description

The different ratios of DBBM and PAB displayed various stages of integration with the host bone after 10 weeks, 20 weeks and 30 weeks of graft healing. Specimens containing different ratios of DBBM and PAB showed DBBM particles embedded in mineralized bone and NMT at all time points. DBBM particles adjacent to the host bone were generally more integrated in mineralized bone compared to particles located adjacent to the periosteum. At 10 weeks of healing, limited amount of ingrowth of mineralized bone into the augmented area was observed, whereas almost complete ingrowth of mineralized bone was seen after 20 weeks and complete mineralization

could be observed in most of the specimens after 30 weeks of healing, regardless of the ratio of DBBM and PAB.

The transition zone between the graft and the host bone are presented in figure 5.

Quantitative histomorphometry

The mean POB, the mean percentage of NMT and the mean percentage of DBBM after 10 weeks, 20 weeks, and 30 weeks, respectively for all ratios are presented in figures 6, 7 and table 2.

Percentage of bone:

The mean POB for the different ratios used during GBR were 54% (50:50), 50% (75:25) and 48% (100:0) after 10 weeks, 60% (50:50), 61% (75:25) and 60% (100:0) after 20 weeks and 63% (50:50), 62% (75:25) and 62% (100:0) after 30 weeks.

The POB were similar for all ratios at all time points and no statistically significant differences could be detected ($p>0.05$). However, there was a significant increase in POB from 10 weeks to 30 weeks for 75:25 ($p=0.02$) and 100:0 ($p=0.01$)

Percentage of NMT:

The mean percentage of NMT for the different ratios were 25% (50:50), 27% (75:25) and 26% (100:0) after 10 weeks, 18% (50:50), 15% (75:25) and 15% (100:0) after 20 weeks and 22% (50:50), 16% (75:25) and 16% (100:0) after 30 weeks.

The amount of NMT were similar for all ratios at all time points and no statistically significant differences could be detected ($p>0.05$). However, there was a significant decrease in percentage of NMT from 10 weeks to 30 weeks for 75:25 ($p=0.05$) and 100:0 ($p=0.04$).

Percentage of DBBM:

The mean percentage of DBBM for the different ratios were 21% (50:50), 23% (75:25) and 26% (100:0) after 10 weeks, 22% (50:50), 24% (75:25) and 25% (100:0) after 20 weeks and 16% (50:50), 22% (75:25) and 22% (100:0) after 30 weeks.

Quantitative histology

The ingrowth of mineralized bone into the augmented area with different ratios of DBBM and PAB at different time points is presented in figure 8. The mean ingrowth of mineralized bone into the graft for the different ratios were 44% (50:50), 55% (75:25) and 36% (100:0) after 10 weeks, 89% (50:50), 88% (75:25) and 82% (100:0) after 20 weeks and 99% (50:50), 100% (75:25) and 96% (100:0) after 30 weeks. The values were significantly higher after 30 weeks compared to 10 weeks for all ratios ($p = 0.001$) but no significant difference between the different ratios could be detected ($p>0.05$).

DISCUSSION

Preclinical trials for assessment of bone regeneration with grafting materials have previously been conducted in minipigs (Jensen et al.; Jensen et al., 2013; Jensen et al., 2012). Previous experimental studies assessing different ratios of DBBM and PAB have mainly assessed bone formation in the early healing phase (8-12 weeks), to provide information regarding biocompatibility of the material, early cellular response and persistence and condition of the transplant within the defect. In clinical settings, implants are usually placed 7 to 10 months after LRA (Hammerle et al., 2008; Meloni et al., 2017; Mendoza-Azpur et al.; Mordenfeld et al., 2017; Urban et al., 2011; Urban et al., 2013). Histomorphometrical and histological assessment after a prolonged healing period and at different time points could provide valuble knowledge on bone regeneration and incorporation of the graft material prior to implant placement. The present study included two healingperiods that corresponds to clinical settings where mineralization and incorporation of the graft material, the quantity of the material and placement of the evaluated material is comparable to the clinical situation for LRA. To the best of the authors knowledge, no preclinical studies have assessed the regenerative outcome for different ratios of DBBM and PAB in the lateral situation over time.

Although the anatomy, bone mineral density, bone remodeling rate and healing potential of minipigs are comparable to humans (Reinholz, Lu, Saris, Yaszemski, O'Driscoll, 2004), the results of the present preclinical trial should be interpreted with caution since lateral augmentation was performed outside the skeletal envelope and in a location on the lateral aspect of the mandible, where a possible periosteal driven bone formation should be taken into account. However, this is equally distributed between the respective groups. Another possible side effect to bear in mind is

the postoperative behavior of the animals with difficulties to control pressure to the augmented area.

Even though there will be a degradation of the collagen membranes, the induction and organization in bone formation following GBR has already taken place. Resorbable collagen membranes are widely used and are well-documented in the clinical situation where the initial barrier function seems to be enough to achieve bone formation for lateral augmentation (Mendoza-Azpur et al.; Mordenfeld et al., 2017; Urban et al., 2011; Urban et al., 2013), why the membrane was considered as suitible for the present study.

In the present randomized controlled trial in minipigs, lateral GBR augmentation of the mandible with different ratios of DBBM and PAG was assessed by histological and histomorphometrical measurements after 10 weeks, 20 weeks and 30 weeks, respectively. There were no significant differences in POB, percentage of NMT or percentage of DBBM at any time point, regardless of the ratio of DBBM and bone.

The POB increased significantly between 10 and 30 weeks on the expense of NMT. After 30 weeks, a complete mineralization of the grafts was observed. This is in accordance with a previous statement, that bone is formed with an approximate rate of 1 mm per month (Schenk, Buser, Hardwick, Dahlin, 1994). According to the presented results, it seems like a prolonged healing period will have a beneficial effect on the incorporation and mineralization of the grafts.

Previous studies on lateral augmentation by GBR with different compositions of DBBM and PAB have placed implants 7 to 10 months after the augmentation procedure with satisfactory results (Hammerle et al.; Meloni et al., 2017; Mendoza-Azpur et al.; Mordenfeld et al.). This seems to be a sufficient healing period according to the bone formation presented in this study. However, a conclusion on when to continue with the subsequent implant treatment in the clinical situation cannot be drawn from the results in the present preclinical trial.

The percentage of DBBM maintained without any significant changes during the entire observation period, indicating the absence of resorption of the bone substitute These findings are in accordance with results from previous studies (Mordenfeld, Hallman, Johansson, Albrektsson, 2010). However, in the literature there is a controversy regarding whether DBBM is a resorbable material or not (McAllister et al., 1999; Simion, Fontana, Rasperini, Maiorana, 2007; Tadjoedin, de Lange, Bronckers, Lyaruu, Burger, 2003).

The regenerative outcome after augmentation with DBBM and PAG has previously been assessed in standardized defects and after augmentation of the maxillary sinus in preclinical

studies. In the early healing phase, an increased formation and maturation of bone has been observed after grafting procedures with PAB alone compared to DBBM alone (Burchardt, 1983; Jensen et al.). Analyses of bone-to-implant contact with different ratios of DBBM and PAG demonstrated an increased bone-to-implant contact with PAG alone compared to DBBM alone, but the bone-to-implant contact was not significantly influenced by the ratio of DBBM and PAG (Jensen et al., 2013). These findings could not be confirmed in the present study, since no significant difference regarding bone formation was observed between different ratios of DBBM and PAG, neither in the early, nor in the late healing phase. The discrepancies in the results could depend on the differences in the biological environments and demands on mechanical stability after LRA, compared to augmentation in standardized defects and after augmentation in the maxillary sinus. Standardized defects mainly comprise of at least two walls to support the augmented area, while the maxillary sinus is a closed compartment where no pressure will be applied after the augmentation procedure. For LRA, the procedure may demand a material with high mechanical stability and low resorption rate due to the pressure from the overlying mucosa, cheeks and lips and osteoinductive properties to enhance bone formation. However, the present study did not demonstrate any enhanced bone formation nor incorporation of the graft with addition of autogenous bone to the DBBM for lateral augmentation.

A previous histomorphometrical study has demonstrated that in a graft consisting of 100% DBBM, the DBBM particles only occupy 43.5% of the total area at the moment of grafting and the remaining 56.6% is NMT, even after a firm compression of the graft (Aludden, Dahlin, Starch-Jensen, Dahlin, Mordenfeld, 2017). Hence, there is space enough for bone ingrowth between the DBBM particles if soft tissue ingrowth is occluded by a membrane due to the principles of GBR.

Stability of the graft during the consolidation period is an important factor for the regenerative outcome. Clinical studies have demonstrated a superior increase in the width of the alveolar process when the graft material was stabilized under a membrane fixed with titanium pins compared to no fixation (Mendoza-Azpur et al.; Mordenfeld et al.; Urban et al.; Urban et al.). These findings have also been confirmed in a radiological study in minipigs (Mir-Mari, Wui, Jung, Hammerle, Benic, 2016). A firm and stable graft will probably counteract compression of the augmented area and might be more important than the addition of autogenous bone for lateral GBR augmentation. Bone formation has previously been presented only by using the principles of GBR, without addition of autogenous bone (Simionet al., 2007).

The results of the present experimental study are influenced by confounding factors and limitations as the specific animal model, the method of augmentation and the chosen healing periods.

The healing periods of the present study were chosen to simulate healing periods, frequently used for lateral GBR procedures in humans. However, by not in including shorter healing periods, a possible stimulatory effect of autogenous bone on the bone formation in the early healing phase cannot be evaluated. Randomized controlled trials in humans are therefore needed, before definitive conclusions can be provided for lateral GBR augmentation with DBBM alone or in combination with PAB.

CONCLUSION

Within the limitations of the present study, it seems like addition of autogenous bone to DBBM for LRA did not affect the bone formation nor graft incorporation after 10 to 30 weeks of healing. However, a prolonged healing time seems to result in an increased POB for all ratios. Clinical randomized controlled trials assessing lateral ridge augmentation with different ratios of DBBM and PAG are needed before well-defined conclusions can be provided.

CONFLICT OF INTEREST

Dr. Hanna Aludden has received Bio-Oss and Bio-Gide membranes from Geistlich Pharma and Frios titanium pins from Dentsply Sirona to perform the present study.
Furthermore, Geistlich pharma has supported an ongoing clinical trial with Bio-Oss and Bio-Gide membranes.

AUTHOR CONTRIBUTION

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TABLE 1. Number of specimens for histological analysis

	10 weeks	20 weeks	30 weeks
50:50	8	8	7
75:25	8	7	7
100:0	8	7	8
ABB	7	7	8

Abbreviations: ABB: Autogenous bone block; 50:50: 50% deproteinized bovine bone mineral 50% particulated autogenous bone; 75:25: 75% deproteinized bovine bone mineral 25% particulated autogenous bone; 100:0: 100% deproteinized bovine bone mineral

TABLE 2. The mean percentage (%) of bone, non-mineralized tissue and deproteinized bovine bone mineral

Graft material	Consolidation period												
	10 weeks			20 weeks					30 weeks				
	Bone (%) (CI)	NMT (%) (CI)	DBBM (%) (CI)	Bone (%) (CI)	p value	NMT (%) (CI)	p value	DBBM (%) (CI)	Bone (%) (CI)	p value	NMT (%) (CI)	p Value	DBBM (%) (CI)
50:50	54 (42-62)	25 (21-29)	21 (14-23)	60 (54-65)	0.30	18 (14-23)	0.20	22 (17-25)	63 52-73	0.12	10-33 16	0.7	16 8-22
75:25	50 (43-58)	27 (19-34)	23 (15-25)	61 (50-71)	0.06	15 (7-24)	0.02	24 (17-30)	62 51-73	0.02	7-26 22	0.05	22 17-24
100:0	48 (45-52)	26 (20-33)	26 (20-27)	60 (48-71)	0.05	15 (3-27)	0.02	25 (18-29)	62 57-67	0.01	10-22 22	0.04	22 (14-30)
ABB	90 (87-95)	10 (7-13)		91 (88-95)	0.99	9 (5-12)	0.99		95 93-97	0.6	3-7	0.7	

Abbreviations: ABB: Autogenous bone block; CI: 95% confidence interval; DBBM: Deproteinized bovine bone mineral; NMT: Non-mineralized tissue; 50:50: 50% deproteinized bovine bone mineral 50% particulated autogenous bone; 75:25: 75% deproteinized bovine bone mineral 25 % particulated autogenous bone; 100:0: 100% deproteinized bovine bone mineral; p-value: the p-value from baseline to 20 and 30 weeks respectively.

Figure 1: Lateral aspect of the mandible demonstrating position screws and cortical perforations at the 4 recipient sites

Figure 2: A prefabricated stainless-steel frame to ensure a standardized amount of graft material at each recipient site

Figure 3: Illustration of the four augmented areas

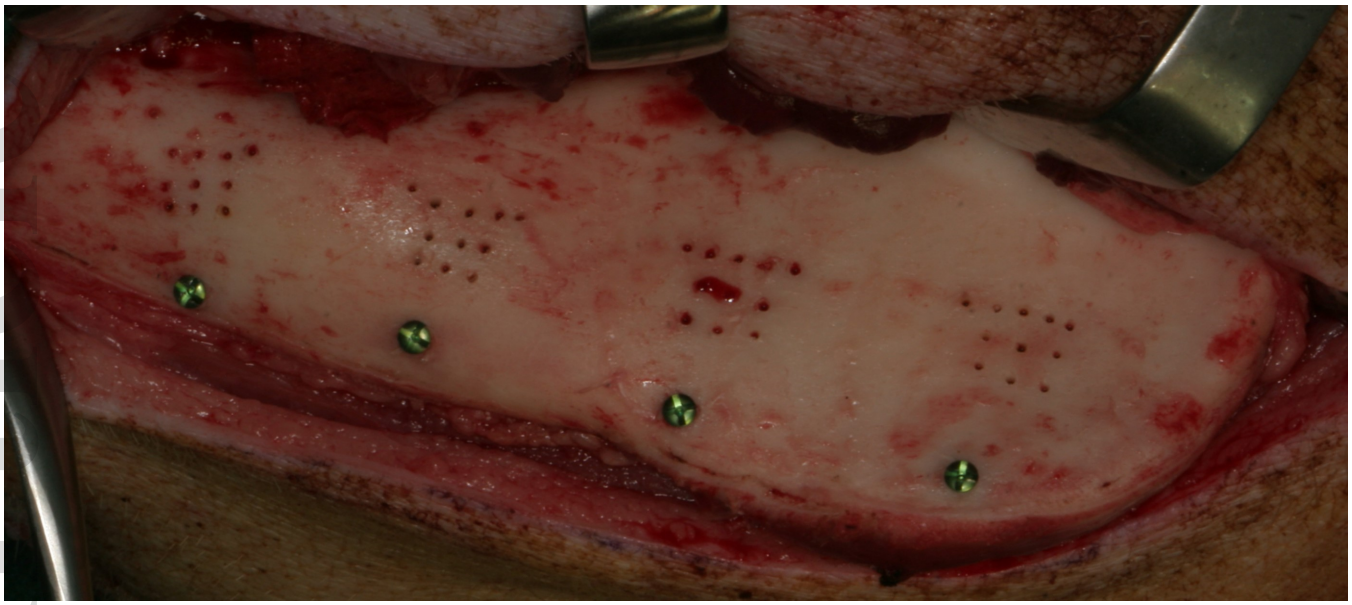
Figure 4: Illustration of the measurements for ingrowth of mineralized bone into the graft

Figure 5: Illustration of the transition zone between the graft and the hostbone for a graft consisting of 100% deproteinized bovine bone mineral after 20 weeks of healing

Figure 6: Illustration of the percentage of bone for the different ratios over time

Figure 7: Illustration of the percentage of non-mineralized tissue for the different ratios over time

Figure 8: Illustration of the ingrowth of mineralized bone into the graft for the different ratios over time



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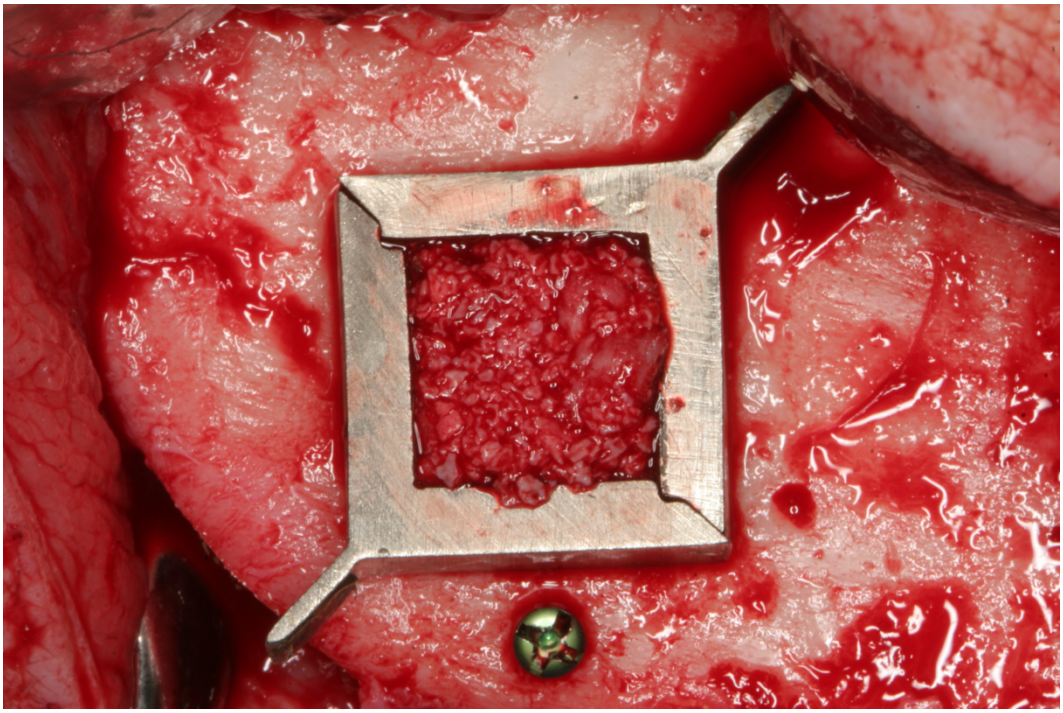

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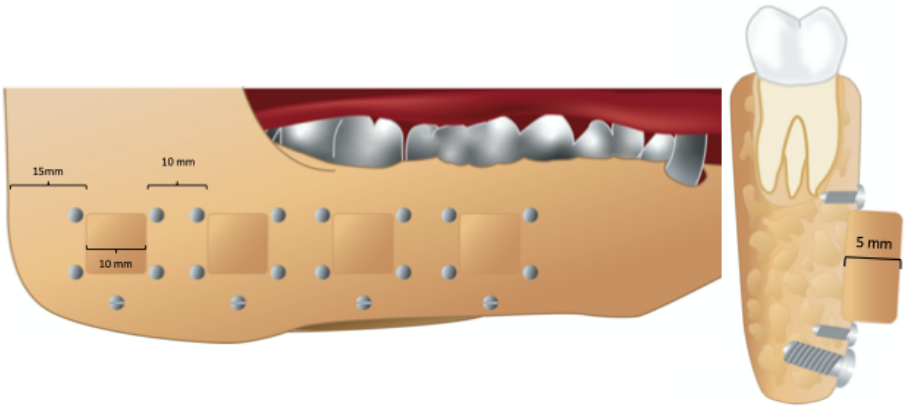

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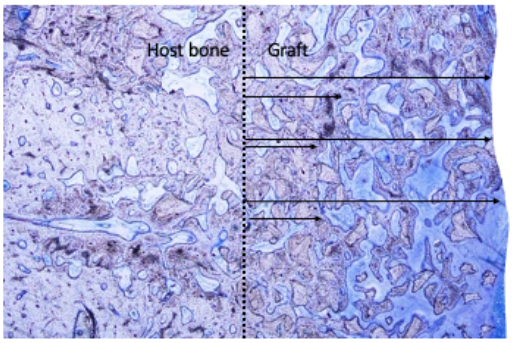

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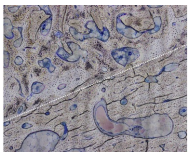

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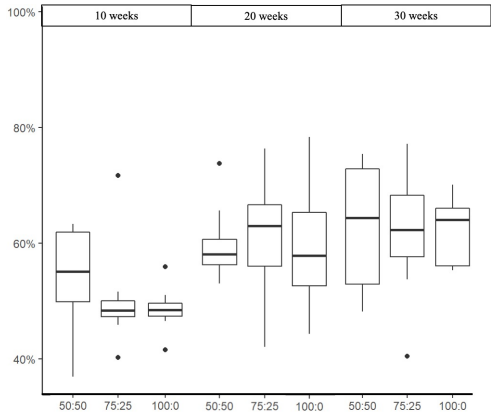

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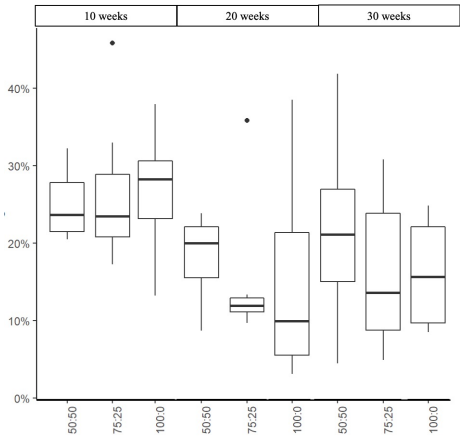

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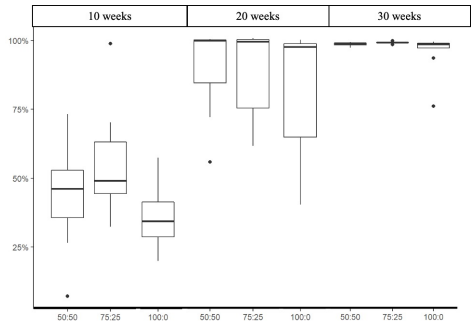

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