CLINICAL ORAL IMPLANTS RESEARCH

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Bone tissue modelling and remodelling following guided bone regeneration in combination with biphasic calcium phosphate materials presenting different microporosity

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Abstract

Objectives: The aim of this study was to investigate bone regeneration following application of a novel biphasic calcium phosphate (BCP I) composed of microstructured granules of 90% β-tricalcium phosphate (β-TCP)/10% hydroxyapatite (HA) compared to BCP non-microstructured biphasic calcium phosphate with a composite of 60% hydroxyapatite/40% β-TCP (BCP II) and a deproteinized bovine bone mineral (DBBM) at surgically created defects in the mandible of minipigs in a combined approach with guided bone regeneration (GBR).

Material and methods: Sixteen minipigs were used for the study. Lower premolars P2, P3, P4 and first molar M1 were extracted. Following 3 months of healing, two defects with a width and depth of 7 mm were created bilaterally in the mandible. The different grafting materials were randomly placed in the created defects and covered by means of a collagen membrane. After 3 and 8 weeks, biopsies were sampled. All specimens were evaluated with descriptive histology and histomorphometric evaluations complemented by micro-CT scan analysis.

Results: All three biomaterials presented with higher bone volume at 8 weeks compared to 3 weeks ($P < 0.0442$). BCP I and DBBM demonstrated a significant higher amount of bone formation compared to BCP II at 8 weeks ($P < 0.0328$). BCP I also demonstrated a significant higher percentage of remaining graft volume compared to the other test groups both at 3 and 8 weeks ($P < 0.0001$ to $P < 0.0003$). Congruently, defects containing BCP I showed a significant higher amount of mineralized tissue compared to the other groups.

Conclusions: All the three test materials performed well with regard to bone formation at 8 weeks. BCP I showed significant higher amounts of newly formed bone despite a higher remaining graft volume compared to the other groups. With regard to the regenerative outcome, all the three materials can be recommended for clinical use.

Over the last two decades, the development of the concept of guided bone regeneration (GBR) has had a significant impact on aesthetic reconstruction in conjunction with oral implant therapy (Dahlin et al. 1988, 1989; Retzepi & Donos 2010). The concept implies that ingrowth of non-osteogenic soft tissue cells is prevented from entering the osseous defect and allowing angiogenic and osteogenic cells from the surrounding bone to migrate into the defect undisturbed. As mentioned by the previous authors, clear synergistic effects have been obtained when barrier membranes were combined with various types of filling materials within the wound defect.

As a filling material in conjunction with GBR procedures, a transition from the use of autogenous bone grafts towards an increased use of various bone graft substitutes has been noted (Froum et al. 1998; Piatelli et al. 1999; De Leonardis & Pecora 2000; Szabo et al. 2001; Wallace & Froum 2003; Hallman & Zetterqvist 2004; Wang et al. 2004; Esposito et al. 2006). Biphasic calcium phosphate (BCP), in a first generation, has been widely used as a bone graft substitute material mainly in orthopedics. Developments of calcium phosphate ceramics and other related biomaterials as bone grafts give the opportunity for an improved control of the process of biomaterial degradation and subsequent bone formation

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and substitution. With the increased awareness and use of dental implants, there is a demand for treatment of more complex cases where predictable grafting prior to or in conjunction with the implant placement is indicated. Various grafting materials have been utilized for ridge augmentation and sinus floor elevation. Autogenous bone (AB), allografts, xenografts, alloplastic materials and mixtures of various components have been evaluated (Del Fabbro et al. 2004; Chiapasco et al. 2006; Mardas et al. 2010).

Biphasic combinations of HA and β-TCP combine the advantages and properties of both materials, providing an excellent balance to achieve long-term stability and new bone formation (Nery et al.1992). It has been suggested that osteoconductivity and dissolution are enhanced; the HA should provide an initial bone response, while the β-TCP provides bone remodelling. The calcium phosphate ceramic granules are dissolved and resorbed while retaining a suitable scaffold for ingrowth of new bone formation, creating a suitable microenvironment for simultaneous osteoblastic activity and allowing effective bone ingrowth and regeneration (Frayssinet et al. 1993; Daculsi 1998).

Pre-clinical and clinical studies with BCP of the first generation have been conducted for over 15 years. The synthesis of biphasic calcium phosphate allows the combination of the desirable characteristics of its components together in one device, so that the biological properties can be adapted to the requirements. Material characteristics such as the composition, crystallinity, macro- and microporosity influence the bioactive properties of BCP. The material is usually prepared by sintering at high temperatures. The ratio of HA to β-TCP is dependent on such factors as the calcium deficiency of the unsintered apatite, the temperature of sintering, the time and the pH value of the precursor. Hence, bioreactivity can be altered by manipulation of the material characteristics (Yuan et al. 1999, 2006a,b).

More recent BCP materials appear to present an interesting configuration related to its HA/TCP (60%:40%) ratio (Jensen et al. 2007, 2009; Mardas et al. 2010). The 60% HA ratio facilitated long-term volume stability but was decelerating the overall resorption capabilities. Histological evaluation demonstrated that BCP promoted osteoblastic and osteoclastic activity characterizing a bone remodelling process and making evident the importance of the HA/TCP ratio in the bony ingrowth (Jensen et al. 2007, 2009). Comparatively to different bone grafts, BCP revealed substantially higher osteogenic capacity, and it seemed to be as safe and efficient as autogenous bone grafts (Fellah et al. 2008).

The aim of the present study was to evaluate comparatively biphasic calcium phosphate materials with different configurations in a GBR setting with regard to new bone formation in a two- and three-dimensional aspect.

Material and methods

Material characteristics

The following bone substitute materials of comparable granular size were used:

1. A composite of 10% hydroxyapatite and 90% β-tricalcium phosphate (BCP I). The particle size 500–1000 μm. Sintered temperature approximately 1000°C (Straumann AG, Basel, Switzerland) (Fig. 1a).
2. A composite of 60% hydroxyapatite (100% cristalline) and 40% β-tricalcium phosphate (BCP II). Sintered temperature approximately 1.500°C. Particle size 500–1000 μm. (Straumann AG) (Fig. 1b).
3. Control. Xenogenic hydroxyapatite particles (BioOss®; Geistlich Biomaterials, Wolhusen, Switzerland) (DBBM). Particle size 250–1000 μm. (Fig. 1c).

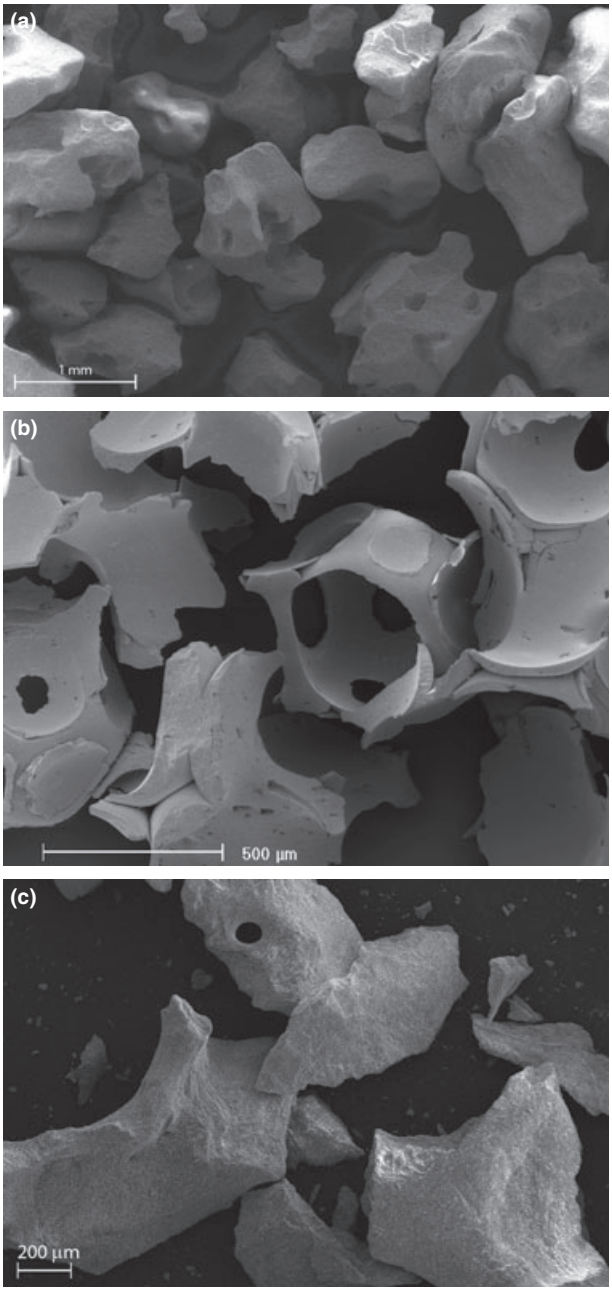

Fig. 1. (a) SEM images of BCP I (500–1000 μm), (b) BCPII (500–1000 μm) and (c) DBBM (250–1000 μm) particles. Note the different configuration.

Barrier membranes for guided bone regeneration

All defects were covered with a collagen membrane (BioGide®; Geistlich Biomaterials).

Experimental model

A total of 16 Göttingen minipigs (Ellegaard, Denmark) were recruited for the trial.

The animal study received an ethical approval from the local IACUC (number 121/08, Lund University, Sweden) and was conducted according to the most established guidelines (Dard 2012).

The animals were kept in specially designated areas under supervision of veterinarian staff during the entire research period. Prior to surgery, the animals were fasted. On the day of surgery, the animals were weighed and pre-medicated with an intra-muscular injection of atropine (0.05 mg/kg IM).

Surgical phases

All surgeries were performed under aseptic conditions in a specially designated animal operating theatre.

The surgeries were performed under general anaesthesia. The anaesthesia was initiated by IM injection of ketamine (Ketalar Vet; Pfizer AB, Sollentuna, Sweden) and midazolam (Dormicum®; Roche, Basel, Switzerland) and maintained by additional ketamine and midazolam when needed.

Teeth extraction

After administration of local anesthesia (2% xylocain–adrenaline; ASTRA, Södertälje, Sweden) following a gingival incision, full-thickness mucoperiosteal flaps were raised bilaterally in the mandible. Lower premolars P2, P3, P4 and first molar M1 were carefully extracted. After rinsing with saline, the wounds were closed with single Vicryl 4-0 sutures (Janssen-Cilag AB, Sollentuna, Sweden). Radiographs were taken postoperatively to verify the sites.

Re-entry and defect creation

After 3 months of healing following teeth extraction, using the same anaesthesia regime as described previously, crestal incisions were made and bucco-lingual full-thickness flaps were raised bilaterally. The edentulous osseous ridges were flattened by means of a rotary bur during profuse cooling with saline to obtain a width of at least 10 mm.

Two defects on each side of the mandible (at a distance of >5 mm between them and 4 mm to M2) were created by means of a graded series or spiral burs. The final depth and width were 7 mm. Titanium mini screws (1.5 × 6 mm, Modus®; Medartis AG, Basel, Switzerland) were placed on top of the crest adjacent to the defect to perform accurate measurements and location for the histological analysis (see Fig. 2). The surgical defects were rinsed with saline to remove possible bone debris. By means of a randomized rotary schedule, the four defects in each animal were filled with BCP I, BCP II or DBBM (Fig. 3). Finally, the crestal entrances of all defects were covered with a collagen membrane (BioGide®; Geistlich Biomaterials). The wounds were closed with single 4-0 Vicryl sutures. Digital postoperative radiographs were taken.

The animals received antibiotics for 7 days postoperatively (Streptocillin vet 3–4 ml/animal i.m.; Boehringer Ingelheim, Ingelheim, Germany). They were also given Temgesic® (3–5 ml/animal i.m; Essex Pharma GmbH, Munich, Germany) for 3 days postoperatively.

After 3 and 8 weeks of healing, respectively, block resections of the experimental sites were performed, and the specimen was fixed in 4% buffered solution for 2 weeks prior to histological processing.

Histomorphometric and volumetric (μ-CT) analysis

μ-CT and morphometric analysis

After embedding and prior to sectioning, the samples were scanned by micro-computed tomography (μCT 1000; Scanco Medical AG, Brütisellen, Switzerland).

The extracted data were filtered using a three-dimensional constrained Gaussian filter with finite filter support (one voxel) and filter width ($\delta = 0.8$). The images were then segmented to separate old bone, new bone and the biomaterial from the background (Fig. 4). For each defect, a contour was morphed manually along the defect axis.

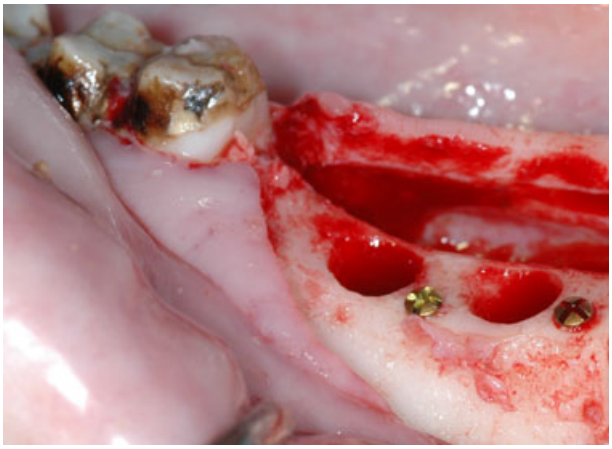

Fig. 2. Clinical photograph illustrating the created standardized socket defects (7 × 7 mm) in edentulous areas of the mini pig. Titanium mini screws were placed for calibration and orientation for histological processing.

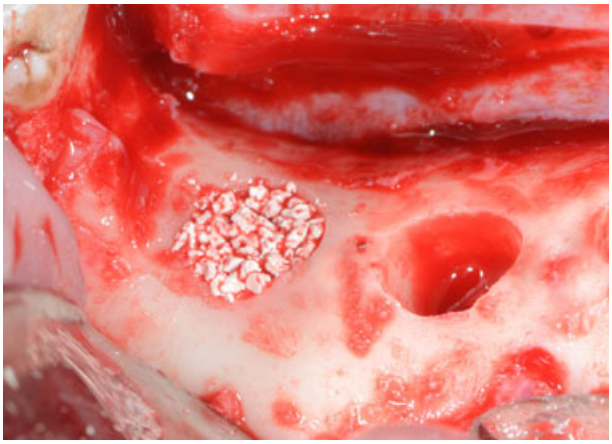

Fig. 3. Experimental socket defect filled with biphasic calcium phosphate (BCP I). The defect will be covered by means of a collagen membrane (BioGide®).

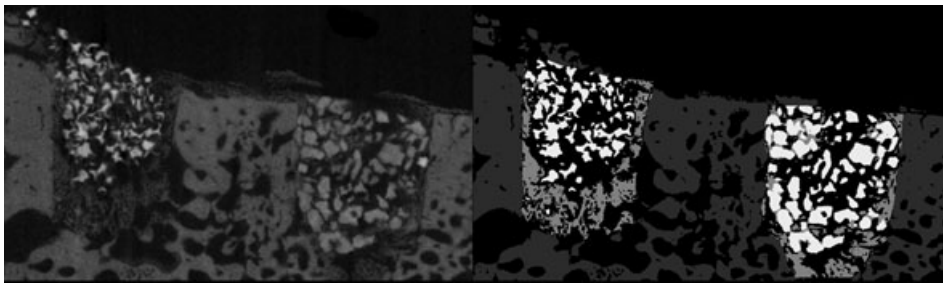

Fig. 4. Photomicrograph of micro CT analysis showing a cross-section of the created defects with biphasic calcium phosphate (BCP II) material in the left and BCP I in the right. Note the new bone formation at the bottom of the defects.

In all defects, the total volume (TV) of the analysed region and the new bone volume (BV) were calculated. From these measures, bone volume density (BV/TV) was computed. (See Tables 1 and 2).

Histomorphometric analysis

Briefly, the bone samples (hemi mandibles) each were immersed in formalin buffer solution, dehydrated using ascending grades of alcohol and xylene, infiltrated and embedded in methylmethacrylate for non-decalcified sectioning. Each defect was cut in bucco-lingual direction. With the help of measurements from the reference screws and postoperative radiographs, the centre of the respective defects was estimated. Each site was cut in a bucco-lingual direction. One representative section per defect of 500 μm was obtained and grinded to a final thickness of 50 μm and stained with paragon (toluidine blue and basic fuchsine) for descriptive histological evaluation and morphometric analysis.

A region of interest (ROI) was defined as the full widths and lengths of the cavity whereby the upper line defined the most outer rim of the bone trabeculae on the top of the alveolar ridge. Photographs were taken from all samples per block by a ColorView IIIu at a magnification lens ×125. Image analysis was performed by CELL Analysis.

Statistical analysis

Outcome parameters from the μ-CT and histomorphometric analyses were summarized in terms of means, standard deviations, medians and quartile values. The two BCP I treatments within an animal were paired by position to the BCP II and to the DBBM treatments. Comparisons between treatments were first examined using the Wilcoxon

Table 1. **Descriptive statistics for the volumetric (μ-CT) analysis by treatment and endpoint**

			Treatment		
Outcome	Endpoint	Parameter	BCP I	BCP II	DBBM
Total volume (mm^3)	3 weeks	*n*	16	8	8
		Mean ± SD	209.8 ± 24.9	215.2 ± 28.8	210.2 ± 25.2
		Median (Q1–Q3)	202.9 (195.6–223.1)	210.8 (189.8–236.1)	199.8 (191.2–231.8)
	8 weeks	*n*	16	8	8
		Mean ± SD	194.7 ± 59.1	195.7 ± 30.73	189.2 ± 42.46
		Median (Q1–Q3)	177.5 (157.1–215.5)	196.1 (169.4–203.6)	171.7 (158.8–225.9)
Bone volume (mm^3)	3 weeks	*n*	16	8	8
		Mean ± SD	27.9 ± 9.5	24.4 ± 9.8	26.8 ± 8.3
		Median (Q1–Q3)	28.2 (20.9–32.6)	19.9 (17.2–30.7)	27.0 (20.6–31.5)
	8 weeks	*n*	16	8	8
		Mean ± SD	63.7 ± 23.4	46.3 ± 20.7	60.3 ± 14.2
		Median (Q1–Q3)	64.2 (45.4–66.5)	45.9 (34.8–63.4)	57.6 (48.8–68.7)
Bone volume density (%)	3 weeks	*n*	16	8	8
		Mean ± SD	13.2 ± 4.3	11.5 ± 4.9	13.0 ± 4.8
		Median (Q1–Q3)	12.3 (10.6–15.65)	9.5 (8.9–14.7)	11.5 (9.7–15.9)
	8 weeks	*n*	16	8	8
		Mean ± SD	32.7 ± 7.7	23.9 ± 10.5	32.1 ± 5.0
		Median (Q1–Q3)	32.7 (25.8–40.8)	24.5 (19.1–31.2)	30.5 (29.0–34.0)

BCP, biphasic calcium phosphate; DBBM, deproteinized bovine bone mineral.

Table 2. **Descriptive statistics for the histomorphometric analysis by treatment and endpoint**

			Treatment		
Outcome	Endpoint	Parameter	BCP I	BCP II	DBBM
Graft (%)	3 weeks	*n*	16	8	8
		Mean ± SD	45.4 ± 7.0	28.8 ± 10.9	26.9 ± 3.4
		Median (Q1–Q3)	45.5 (36.0–59.8)	29.4 (11.0–48.3)	26.3 (22.1–33.9)
	8 weeks	*n*	16	8	8
		Mean ± SD	38.3 ± 9.0	24.8 ± 5.9	22.0 ± 13.0
		Median (Q1–Q3)	41.1 (18.2–48.8)	26.3 (12.9–32.1)	20.8 (1.8–39.5)
Mineralized tissue (%)	3 weeks	*n*	16	8	8
		Mean ± SD	59.2 ± 8.8	40.6 ± 8.0	45.9 ± 6.7
		Median (Q1–Q3)	57.475 (42.7–73.0)	40.7 (31.3–55.8)	46.1 (33.5–57.0)
	8 weeks	*n*	16	8	8
		Mean ± SD	74.5 ± 10.2	60.6 ± 7.2	65.4 ± 15.3
		Median (Q1–Q3)	76.6 (47.4–88.7)	57.9 (52.5–73.9)	63.2 (42.3–84.4)

BCP, biphasic calcium phosphate; DBBM, deproteinized bovine bone mineral.

signed rank test (results not shown). Non-parametric mixed model regressions Brunner–Langer method (Brunner & Langer 1999) for the factors positions, side, age of the animal (as fixed effects) and the factor animal (as a random effect) were carried out to determine the respective comparisons of the different treatment. These regressions were performed separately for each endpoint. A global comparison (Brunner–Langer, F1_LD_F1 model) was also examined, which confirmed the overall effect of the treatments and the time (endpoints) and the lack of interaction between treatment and time. Results from the above regressions were adjusted for multiple comparisons using Dunnett–Hsu's correction.

The level of significance was set at $P < 0.05$. SAS release 9.3 (SAS Institute, Inc. Cary, NC, USA) was used to perform the statistical analysis.

Results

The postoperative period remained uneventful, and all animals and all respective defect sites were available for analysis.

Volumetric analysis (μ-CT)

Table 1 shows the descriptive statistics for TV, BV and BV/TV. Overall, the mean TV ranged from 189–215 mm^3 in the created defects. The BV and the relative BV/TV (bone volume) were higher at 8 weeks than at 3 weeks.

At 3 weeks, and taking into account the effect of the animals, mandible side, position of the defect in the mandible and the age of the animals, the adjusted mean BV/TV was 11.7% for BCP I, 10.6% for BCP II and 11.6% for DBBM, and their differences were statistically not significant. At 8 weeks, the adjusted mean BV/TV was 62.0% for BCP I, 49.0% for BCP II and 61.3% for DBBM. The *P*-value for the comparison of BCPI to BCP II was 0.0328 and 0.8749 for the comparison of BCPI to DBBM. The total volume was similar for all the three treatments at 3 and at 8 weeks.

The *P*-values for the global comparison for the factors treatment, time and interaction of treatment with time were, respectively, 0.0725, 0.0056 and 0.9363 for TV; 0.0986, <0.0001 and 0.5642 for BV; and 0.0132, <0.0001 and 0.3343 for BV/TV.

Descriptive histology

In general, in all materials, the graft particles were well integrated with various degrees of active modelling and remodelling taking place between the 3- and 8-week period.

Three weeks

Both the DBBM and the BCP I particles demonstrated a thin layer of *de novo* bone formation on their respective surfaces. There was a presence of blood vessels in conjunction with the BCP I particles. The BCP II did not exhibit any of the features mentioned above at 3 weeks. The DBBM particles did not show osteoclast activity along the surfaces of the material. In conjunction with the BCP I material, signs of dissolution could be noted. Furthermore, osteoclast activity could be noted at the particle surfaces. In general, more bone formation was also noted in the available space in between the particles for the BCP I (Figs 5–7).

Eight weeks

The DBBM particles were found well integrated with deposition of newly formed bone tissue on the surface of the particles with osteoblast lining and subsequent deposition of osteoid tissue. No evident osteoclast activity could be noted in conjunction with DBBM granules. Hence, no signs of resorption of the particles could be noticed (See Fig. 7). This was in contrast to the BCP I material where ongoing resorption and dissolution of the biphasic calcium phosphate particles was noted with the presence of osteoclasts at the surface of the particles and new bone formation (Fig. 8). At this time point, the BCP II particles demonstrated new bone formation and incorporation with little to moderate resorption.

Histomorphometric analysis

The amounts of graft and mineralized tissue in the defects filled by the three different treatments are shown in Table 2.

The mean amounts of graft at 3 and at 8 weeks adjusted for the effects of animal, side, position and age of the animal are shown for the different treatments in Fig. 9, similar results for the amount of mineralized tissue are given in Fig. 10.

Overall, all the three test groups demonstrated significant increase in bone fill (mineralized tissue) between 3–8 weeks ($P < 0.0442$). Correspondingly, the total amount of graft material was reduced for all groups between 3–8 weeks ($P < 0.0147$). On individual basis, the analysis revealed that the amount of present graft material was significantly higher for BCP I both at 3 and 8 weeks, respectively, compared to the other test materials ($P < 0.0001$ to $P = 0.0003$). Congruently, defects containing BCP I showed a significant higher amount of mineralized tissue compared to the other test groups. BCP II demonstrated the lowest amount of mineralized tissue at 3 weeks. However, at 8 weeks, there were no statistical difference between the BCP II and the DBBM groups.

The *P*-values for the global comparison for the factors treatment, time and interaction of treatment with time were, respectively, <0.0001, <0.0442 and 0.6049 for graft; <0.0001, <0.0001 and 0.6890 for mineralized tissue.

Discussion

In this study, we analysed three materials with regard to *de novo* bone formation in 64

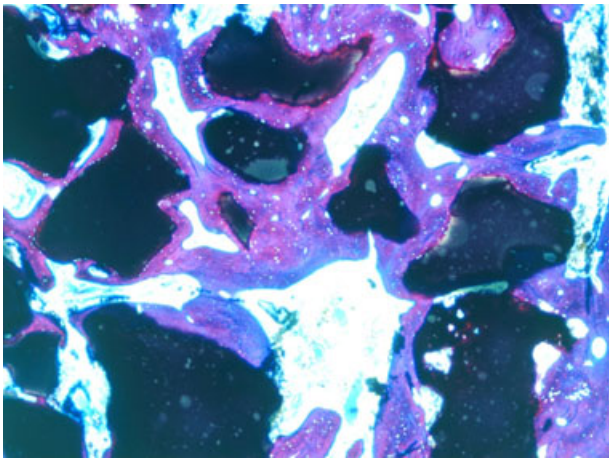

Fig. 5. Microphotograph of mesial–distal section representing an experimental defect filled with biphasic calcium phosphate (BCP I) after 3 weeks of healing. Note active *de novo* bone formation at the particles as well as connecting in the available space. No signs of dissolution of the BCP I particles can be seen. Toluidine blue stain; original magnification ×4.

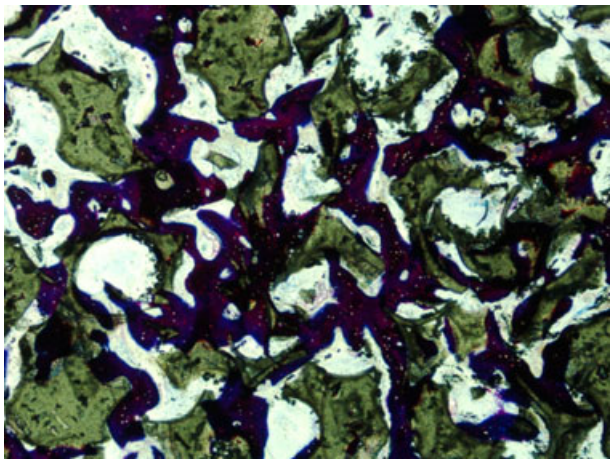

Fig. 6. Microphotograph of mesial–distal section representing an experimental defect filled with biphasic calcium phosphate (BCP II) after 3 weeks of healing. Note minor signs of *de novo* bone formation in between the particles. Paragon stain (toluidine blue and basic fuchsin); original magnification ×4.

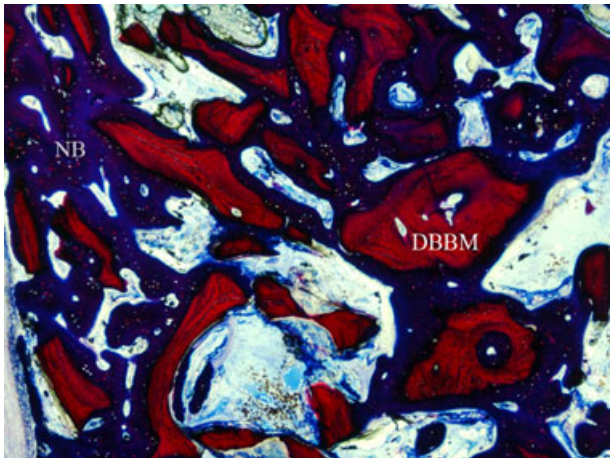

Fig. 7. Microphotograph of mesial–distal section representing an experimental defect filled with deproteinized bovine bone mineral (DBBM) after 3 weeks of healing. Bone deposition is evident on the surface of the particles. Paragon stain (toluidine blue and basic fuchsin); original magnification ×4.

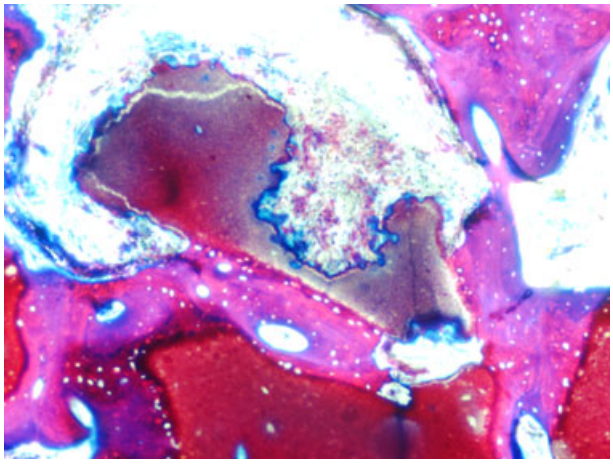

Fig. 8. Higher magnification of biphasic calcium phosphate (BCP I) particles after 8 weeks of healing. Note the active resorption process with osteoclast lacunae of the β-tricalcium phosphate in the centre of the microphotograph. Also note the non-resorbable hydroxyapatite (HA) particle with newly formed bone deposited on the surface at the lower section of the photograph. Paragon stain (toluidine blue and basic fuchsin); original magnification ×10.

standardized drill defects in 32 minipig hemimandibles. Three different biomaterials BCP I, BCP II and DBBM serving as an active control. The selection of animal model for the present study was based on previous experience (Mardas et al. 2014) and the fact that bone tissue in mini pigs has a similar lamellar structure, regeneration and remodelling rate as the human equivalent (Ma et al. 2009). The defects in the present study were not intended to be of critical size and were expected to heal spontaneously without placement of grafting material. Due to the relatively short duration of the experiment, 3–8 weeks, as the aim of the study was to compare between different configurations of grafting materials, the need for a "classical critical size defect" was not considered critical for the outcome of the study. Along the same thought line and furthermore, for ethical reasons, sham defects were not included in the present study to reduce the number of animals involved in the project. Three different biomaterials, BCP I, BCP II and DBBM serving as an active control, were compared. Two of them belonged to the biphasic calcium phosphate group. The principle of GBR was respected by the placement of collagen barrier membranes covering the graft materials during healing. Healing was evaluated at 3 and 8 weeks, respectively. Histomorphometric analysis was the methodological approach of choice complemented by volumetric assessments by means of μ-CT analysis. At 3 weeks, it was noted that both DBBM and BCP I consistently demonstrated new bone formation on the surface of the particles. This could not be noted at this time point for the BCP II group, although the BCP II belongs to the same cluster of materials as BCP I. Histologically, significant more bone formation was noted at 3 and 8 weeks in between the BCP I particles compared to the DBBM material despite the fact that the BCP I group demonstrated almost 10% less available space due to the graft material configuration, hence, providing less available space for bone formation to occur (Fig. 9). Interestingly, this difference was less pronounced at 8 weeks, most probably due to the ongoing resorption and dissolution of the BCP I material (Fig. 8). This pattern could not be noted for the DBBM material where little or no remodelling was found between the respective healing periods. This is in agreement with several authors (Dahlin et al. 2010; Mordenfeld et al. 2010). Surprisingly, at 3 weeks, the BCP II did not exhibit the same features as BCP I. It could be related to the difference in HA/TCP ratio (Jensen et al.

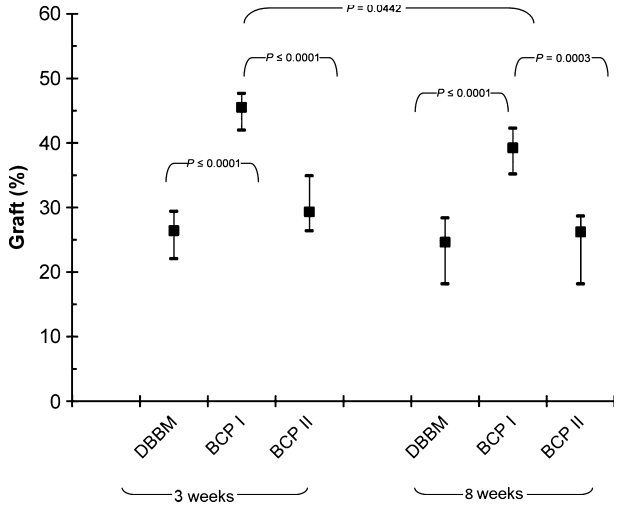

Fig. 9. Histomorphometric analysis; the adjusted means for Graft (%) by endpoint for the respective test groups. The whiskers indicate the 95% confidence intervals.

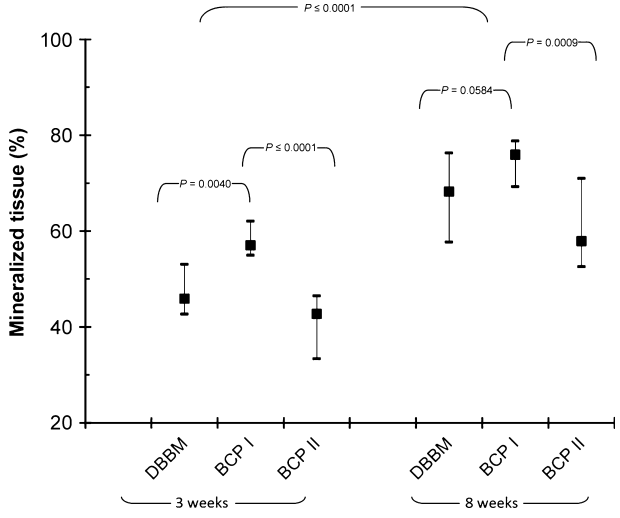

Fig. 10. Histomorphometric analysis; the adjusted means for Mineralized Tissue (%) by endpoint for the respective test groups (graft material excluded). The whiskers indicate the 95% confidence intervals.

2007) or a different surface due to different preparation processes (Habibovic et al. 2006).

The descriptive histological analysis indicated that DBBM was not actively involved in the turnover of the bone tissue and the degradation of particles remained unchallenged by the surrounding hard tissue. Similar findings have been reported in experimental findings by Araújo and Lindhe (2005); Araùjo and Lindhe (2009); Jensen et al. (1996, 2005, 2006); and Artzi et al. (2004). Furthermore, this is also in agreement with the examination of human biopsy material (Evian et al. 1982; Piatelli et al. 1999; Lezzi et al.2008; Mardas et al. 2010; Mordenfeld et al. 2010). This was in marked contrast to the BCP I material where a more active dissolution of the bi-calcium phosphate particles was noted and also a presence of osteoclasts and subsequent resorption (Fig. 8c). *De novo* bone formation was seen in conjunction with this resorption process; however, a clear zone could occasionally be noted between osteoid formation and dissolved areas of the BCP I particles. Reasons for this could be that bone formation occurs with a rather constant rate (Schenk et al. 1994) and the dissolution of the bi-calcium phosphate created a gap in the bone formation adjacent to the particles. Another reason for the gap could be the dissolution of ions in the tissue. It is known that protein adsorption, surface topography, chemical composition and dissolution–precipitation reactions affect cellular responses to biomaterials (Barrère et al. 2008; Engel et al. 2008; Meirelles et al. 2008) This needs to be further analysed for the specific biomaterials used in the present study. The BCP II material comprised of both a synthetic hydroxyapatite (HA) and a tri-calcium phosphate (TCP) ceramics. BCP I was the only material that demonstrated a statistically significant reduction in volume between the 3- and 8-week period. However, due to the fact that the available space for *de novo* bone formation was approximately 10% less in comparison to DBBM, still providing higher amounts of new bone, BCP I demonstrated great potential and further studies on this type of material are recommended. In numerical values, the differences were relatively small between the materials in particular at 8 weeks. From a clinical perspective, the fact that BCP I to a certain extent resorbs (the TCP part of the material) could theoretically be of clear interest as more space would be available in the defect areas for native bone to grow. Hence, the presumably non-resorbable HA part would act as a scaffold and reinforcement of the newly formed bone while maturating. To have a clearer view of potential differences between the respective materials, longer healing periods should also be implemented and studied.

Barrier membranes were applied in the present study according to the principle of GBR to exclude the influence of soft tissue in the bone regenerating process. Two different categories of bone grafting materials were used in present study in conjunction with the membranes. Both BCP I and BCP II showed signs of resorption due to a dissolution pattern with bone neogenesis as a result while DBBM was not actively involved in the bone turnover and acted more as an osteoconductive platform for bone formation. In both situations, taken into account the different biological pathways, the concept of GBR did not cause any adverse effects. Instead, it led to a predictable regeneration and *de novo* for bone formation as has been well characterized in the literature (Hämmerle & Lang 2001; Retzepi & Donos 2010).

In conclusion, the different biomaterials demonstrated clear but different patterns of involvement in bone modelling and remodelling over time when combined with a collagen barrier according to the GBR concept. If one could extrapolate the outcomes to the clinical setting, they could all probably be recommended for use as augmentative materials and well suited to be part of the GBR principle.

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