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Lateral ridge augmentation using particulated or block bone substitutes biocoated with rhGDF-5 and rhBMP-2: an immunohistochemical study in dogs

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Abstract

Objectives: The aim of the present study was to immunohistochemically evaluate lateral ridge augmentation using a particulated (BOG) or block (BOB) natural bone mineral biocoated with rhGDF-5 and rhBMP-2 in dogs.

Materials and methods: Three standardized box-shaped defects were surgically created at the buccal aspect of the alveolar ridge in each quadrant of eight beagle dogs. After 2 months of healing, the chronic-type defects were randomly allocated in a split-mouth design to either (i) BOG or (ii) BOB biocoated with (a) rhGDF-5 or (b) rhBMP-2, respectively. Uncoated grafts served as controls. After 3 and 8 weeks, dissected blocks were prepared for immunohistochemical [osteocalcin (OC)] and histomorphometrical analysis [e.g. area (mm^2) of new bone fill (BF), newly formed mineralized (MT) and non-mineralized tissue (NMT)].

Results: rhBMP-2 biocoated BOG revealed significantly highest BF and MT values at 3 (upper and lower jaws – UJ/LJ – compared with BOG) and 8 weeks (UJ – compared with rhGDF-5). Biocoating of BOB using both rhGDF-5 and rhBMP-2 resulted in significantly increased MT values at 8 weeks (UJ/LJ – compared with BOB). In all groups, NMT adjacent to BOG and BOB scaffolds revealed pronounced signs of an OC antigen reactivity.

Conclusions: Within the limits of the present study, it was concluded that both rhGDF-5 and rhBMP-2 have shown efficacy; however, their bone regenerative effect was markedly influenced by the carrier.

Nowadays, the therapeutic management of new bone formation remains one of the key issues in oral implantology. The presence of three critical ingredients, i.e. soluble molecular signals, responding cells with associated cell-surface receptors, and assembly of the extracellular matrix have been defined as a pre-requisite in order to predictably achieve this goal (Reddi 1994, 1995). The molecular mechanisms for such regeneration are the osteogenic proteins of the transforming growth factor-β (TGF-β) superfamily, of which the bone morphogenetic proteins (BMP) are a class of powerful inducers of bone differentiation (Wozney et al. 1988; Duneas et al. 1998; Ripamonti & Duneas 1998; Ripamonti 2006). BMP-2, which has been described as an anthelix structure, seems to possess the highest osteoinductive potential among the BMPs (Laub et al. 2001). In recent years, the regenerative potential of recombinant human (rh) BMP-2 has been demonstrated in various experimental animal studies, including sinus floor augmentation, alveolar ridge preservation, bone augmentation procedures, and periodontal repair (Boyne et al. 1997; Sigurdsson et al.

1997; Cochran et al. 1999; Wikesjö et al. 1999; Tatakis et al. 2002; Hanisch et al. 2003; Jung et al. 2003; Jovanovic et al. 2007). Most recently, the beneficial effects of rhBMP-2 have also been demonstrated on the osseointegration of titanium implants in several experimental animal studies (Sykaras et al. 2001; Matin et al. 2003; Becker et al. 2006). Growth/differentiation factor-5 (GDF-5), also known as cartilage-derived morhogenetic protein-1, shows a close structural relationship to BMPs. GDF-5 is also a member of the TGF-β superfamily, sharing BMP receptor type IB with BMP-2/4 (Nishitoh 1997). GDF-5 has been demonstrated to promote mesenchymal cell recruitment and skeletal processes such as endochondral ossification, synovial joint formation, tendon/ligament development, and odontogenesis (Nishitoh et al. 1996; Morotome et al. 1998; Buxton et al. 2001; Archer et al. 2003; Coleman & Tuan 2003; Kuniyasu et al. 2003; Sena et al. 2003; Settle et al. 2003; Shimaoka et al. 2004). In these studies, GDF-5 was delivered with atelocollagen, porous hydroxyapatite ceramic, or dicalcium phosphate. Moreover, mutation in GDF-5 has been associated with skeletal abnormalities in both mice and humans (Storm et al. 1994; Storm & Kingsley 1996). Most recent *in vitro* results revealed that GDF-5 significantly increased the proliferation of primary osteoblasts, periosteum cells, and connective tissue fibroblasts. Newly formed bone- and cartilage-like tissue contained chondrocyte, osteocyte, and osteoclastic cells, and were immunoreactive for both types I and II collagen (Yoshimoto et al. 2006). Furthermore, GDF-5 promoted the osteogenic differentiation of rat fat-derived stromal cells as well as the angiogenic activity of stromal cells by increasing vascular endothelial growth factor gene expression *in vitro* (Zeng et al. 2006). Indeed, the application of a rhGDF-5 biocoated β-tricalcium phosphate (β-TCP) (MD05) resulted in a superior bone regeneration when compared with uncoated bone substitutes (e.g. bovine bone mineral, β-TCP), or unfilled control defects in a rat calvarial defect model. In particular, MD05 was associated with a complete osseous bridging of the defect and with the presence of normal bone marrow (Poehling et al. 2006). However, one potential drawback in this study was the lack of a rhGDF-5 biocoated bovine bone mineral, because β-tricalcium phosphate undergoes rapid degradation and is associated with poor mechanical properties (Bartold et al. 2006). So far, however, there are currently no histological data available evaluating the bone regenerative effect of rhGDF-5 biocoated bovine bone mineral, even though its biocoating with rhBMP-2 has been demonstrated to enhance the maturation process of bone regeneration and increased the graft to bone contact in humans (Jung et al. 2003). Unfortunately, the restricted availability of rhBMP-2 may limit its routine application for bone regeneration in oral implantology. Therefore, the aim of the present study was to immunohistochemically evaluate lateral ridge augmentation using a particulated or block natural bone mineral biocoated with rhGDF-5 and rhBMP-2 in dogs.

Material and methods

Animals

Eight beagle dogs (age 14–18 months, mean weight 12.6 ± 0.8 kg) were used in the study. All animals exhibited a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with soft-food diet and water. Animal selection, management, and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University and the Bezirksregierung Düsseldorf. The experimental segment of the study started after an adaptation period of 4 weeks.

Study design and randomization

The study was performed in two surgical phases. In the first phase, extraction of the mandibular and maxillary second, third, fourth pre-molar as well as first and second molar (P2–M2) was performed bilaterally in each dog. Following tooth extraction, three standardized box-shaped defects were surgically created at the buccal aspect of the alveolar ridge in each quadrant. After 2 months of submerged healing, the chronic-type defects were randomly allocated in a split-mouth design to either (i) a particulated or (ii) a block natural bone mineral biocoated with (a) rhGDF-5, and (b) rhBMP-2, respectively. Uncoated materials served as controls. Randomization was performed according to a computer-generated list (RandList®, DatInf GmbH, Tübingen, Germany). Accordingly, all dogs received each treatment procedure once in the upper and lower jaws (UJ/LJ). The animals were killed after a submerged healing periods of 3 and 8 weeks, including four dogs each.

Surgical Phase 1 (tooth extraction and defect creation)

Following intramuscular sedation with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Düsseldorf, Germany), anaesthesia was initiated using 21.5 mg/kg thiopental-sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). During all surgical procedures, inhalation anaesthesia was performed by the use of oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant rate infusion of lactated Ringer's solution while anesthetized. Intraoperative analgesia was performed by intravenous injection of 0.4 mg/kg piritramid (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofene (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For post-operative treatment, piritramid and carprofene were applied subcutaneously for 3 days in the same dose as described before. Additionally, prophylactic administration of clindamycine (11 mg/kg body weight, Clerobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and post-operatively for 3 days.

In the first surgery, P2–M2 were carefully removed bilaterally in both jaws after reflection of mucoperiosteal flaps and tooth separation. Following tooth extraction, three standardized box-type defects (9 mm in height from the crestal bone, 6 mm in depth from the surface of the buccal bone, and 12 mm in width mesiodistally) were prepared in the buccal bone at a distance of 5 mm with a straight fissure carbide bur. The corresponding lingual bone plates were left intact. The defect sizes were standardized by the use of a periodontal probe (PCP12, Hu-Friedy Co., Chicago, IL, USA) (Fig. 1a). All osteotomy procedures were performed under copious irrigation with sterile 0.9% physiological saline. After wound closure by means of mattress sutures (Resorba®, Nürnberg,

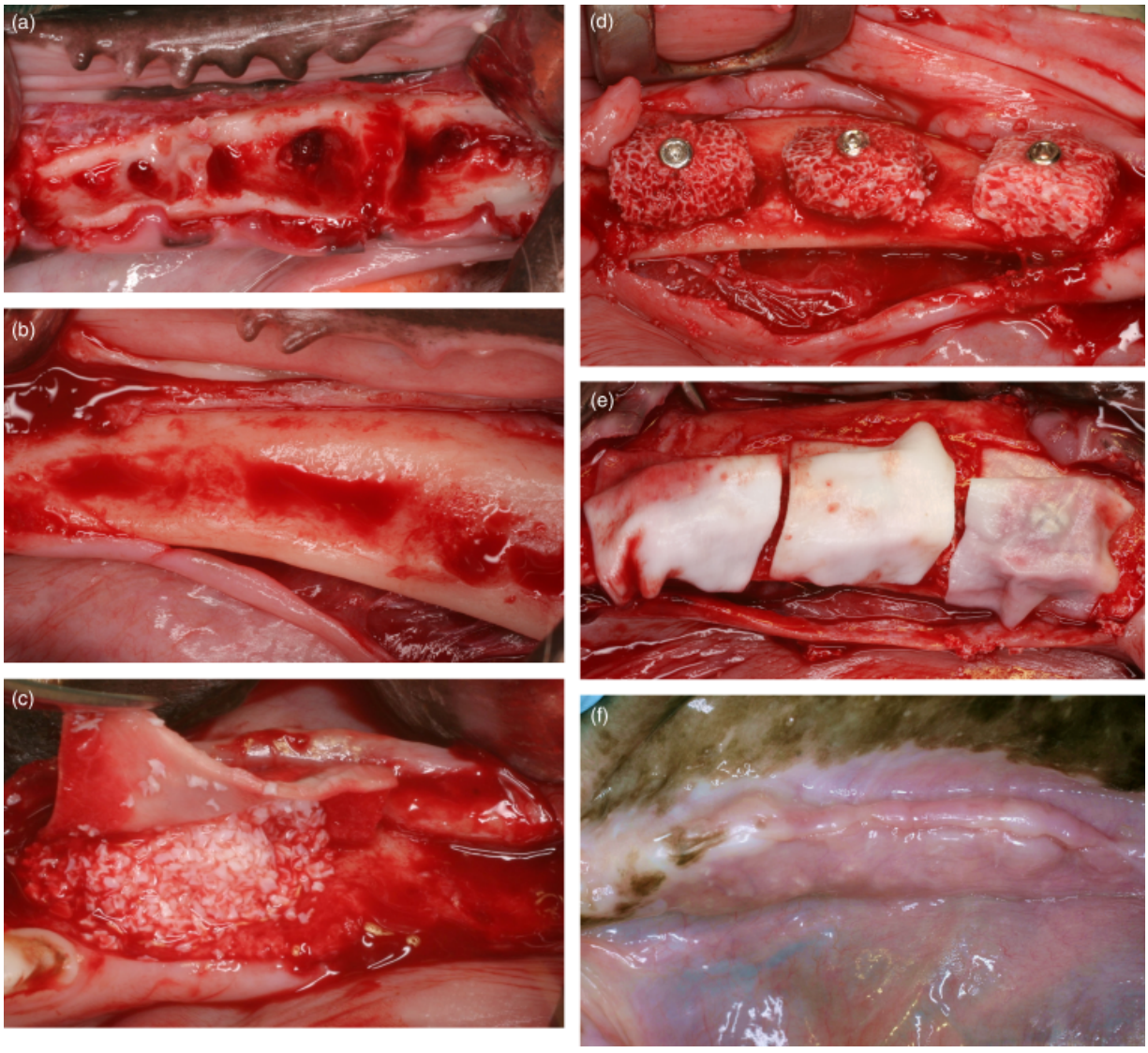

Fig. 1. (a) Standardized box-type defects (9 mm in height from the crestal bone, 6 mm in depth from the surface of the buccal bone, and 12 mm in width mesiodistally; distance between defects: 5 mm) were created at the buccal aspect of the alveolar ridge following tooth extraction. The figure illustrates the defect situation in the lower jaw. (b) Situation after 2 months of submerged healing, showing a chronic-type defect morphology. (c) BOG was applied in a way as to homogeneously fill the complete defect area. (d) BOB was size-adapted (width and height) to the respective sites and fixed by an osteosynthetic titanium screw. The thickness of all blocks was reduced to 6 mm. (e) BG was trimmed and adapted over the entire defect areas in both groups so as to cover 1–2 mm of the surrounding alveolar bone. Neither sutures nor pins were used for membrane fixation or stabilization. (f) The augmented sites were allowed to heal in a submerged position for 3 and 8 weeks. The figure illustrates a non-exposed site (BOB) in the lower jaw after 8 weeks.

Germany), the sites were allowed to heal for 2 months.

Biocoating

The lyophilized growth factors rhBMP-2 (dibotermine α, InductOs®, Wyeth Pharma GmbH, Muenster, Germany) and rhGDF-5 (rhBMP-14, Scil Technology GmbH, Martinsried, Germany) were dissolved in sterile water for injection according to the instructions given by the manufacturer. For biocoating of the particulated graft, 0.5 g BOG was moistened with either 0.67 ml of a 0.77 mg/ml rhBMP-2 solution (1 mg rhBMP-2/g) (Jung et al. 2003), or 0.57 mg/ml of a rhGDF-5 solution (768 μg rhGDF-5/g). The control samples were moistened with 0.67 ml of sterile saline alone.

Similarly, BOB was biocoated with either 2.3 ml of a 0.77 mg/ml rhBMP-2 solution (1.77 mg rhBMP-2 per block) or 0.32 mg/ml of a rhGDF-5 solution (732 μg rhGDF-5 per block), while the control samples were moistened with 2.3 ml of sterile saline alone. The dosages of rhGDF-5 were chosen according to a recommendation given by the manufacturer. Both BOG and BOB were implanted within 30 min upon assembly.

Surgical Phase 2 (bone augmentation)

In the second surgery, bilateral vestibular incisions were made and mucoperiosteal flaps were reflected to expose the respective sites for lateral ridge augmentation in both jaws. Before bone grafting, all granulation tissue was carefully removed from the chronic-type defects (Fig. 1b).

Subsequently, the defects were randomly augmented with either a rhGDF-5/rhBMP-2 biocoated (i) particulated (BioOss® spongiosa granules, particle size 0.25–1 mm, Geistlich Biomaterials, Wolhusen, Switzerland) (BOG), or (ii) a block (BioOss® spongiosa block, width: 20 mm, thickness: 10 mm, height: 10 mm, Geistlich Biomaterials) (BOB) natural bone mineral.

Accordingly, the following sites were tested in both UJ and LJ of each dog:

Site 1: BOG alone	*Site 4*: BOB alone
Site 2: BOG+rhBMP-2	*Site 5*: BOB+rhBMP-2
Site 3: BOG+rhGDF-5	*Site 6*: BOB+rhGDF-5

In particular, BOG was applied in a way as to fill homogeneously the complete defect area (0.25–0.3 g BOG corresponding to 250–300 µg rhBMP-2, or 192–230 µg rhGDF-5 per defect) (Fig. 1c), while BOB was size-adapted (width and height) to the respective sites and fixed by an osteosynthetic titanium screw. The thickness of all blocks was reduced to 6 mm, corresponding to 295–443 µg rhBMP-2, or 122–183 µg rhGDF-5 per defects (Fig. 1d).

Following grafting in both groups, a native bioresorbable collagen membrane of porcine origin (BioGide®, Geistlich Biomaterials) (BG) was trimmed and adapted over the entire defect areas so as to cover 1–2 mm of the surrounding alveolar bone. Neither sutures nor pins were used for membrane fixation or stabilization (Fig. 1c and e). Following periosteal-releasing incisions, the mucoperiosteal flaps were advanced, repositioned coronally and fixed with vertical or horizontal mattress sutures (Resorba®) in a way to ensure a submerged healing procedure.

Retrieval of specimens

The animals were killed after a healing period of 3 and 8 weeks ($n = 4$ dogs each), respectively, and the oral tissues were fixed by perfusion with 10% buffered formalin administered through the carotid arteries. The jaws were dissected and blocks containing the experimental specimens were obtained. All specimens were fixed in 10% neutral buffered formalin solution for 4–7 days.

Histological preparation

The specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated, and embedded in methylmethacrylate (MMA, Technovit 9100 NEU, Heraeus Kulzer, Wehrheim, Germany) for non-decalcified sectioning. During this procedure, any negative influence of polymerization heat was avoided due to a controlled polymerization in a cold atmosphere (– 4°C). After 20 h the specimens were completely polymerized. Serial sections (Exakt®, Apparatebau, Norderstedt, Germany) were prepared from the central parts of the respective defect areas in buccooral direction, resulting in six sections of approximately 500 µm in thickness each (Donath 1985). Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer) to opaque plexiglas and ground to a final thickness of approximately 30 µm. One part of the sections was scheduled for histomorphometrical analysis and stained with toluidine blue (TB), while the other part of the sections was prepared for immunohistochemical labelling.

Immunohistochemical labelling

For immunohistochemistry all tissue section were deplasted in xylol (2 × 30 min) followed by a treatment in 2-methoxyethylacetate (2 × 20 min) and acetone (2 × 5 min). After rehydration in phosphate-buffered saline (PBS), antigen unmasking was performed by incubating the slides for 15 min in trypsin (0.05% in PBS, PAA Laboratories GmbH, Pasching, Austria) at 37°C. After washing with PBS the activity of endogenous peroxidase was quenched with 0.9% hydrogen peroxide in PBS for 10 min at room temperature, the specimens were washed and non-specific binding sites were blocked with a blocking solution for 30 min (DakoCytomation, Hamburg, Germany). The primary mouse monoclonal antibody to osteocalcin (OC) (1 : 40 dilution, Acris Antibodies GmbH, Hiddenhausen, Germany) and the corresponding unspecific antibody (mouse IgG_1), respectively, as negative control were applied to tissue sections in a humidified chamber and incubated overnight at 8°C. The slides were washed in PBS and incubated with secondary biotinylated anti-mouse antibody (1 : 50 dilution, Dako Cytomation, Hamburg, Germany) for 90 min at room temperature. After washing in PBS the presence of antibody–antigen complexes was visualized using a streptavidin-peroxidase solution (1 : 250 dilution, Dako) and AEC (3-amino-9-ethylcarbazole) as the chromogen (Dako).

Histological analysis

Histomorphometrical analyses as well as microscopic observations were performed by one experienced investigator masked to the specific experimental conditions. For image acquisition a color CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus). Digital images (original magnification × 200) were evaluated using a software program (analySIS FIVE docu®, Soft Imaging System, Münster, Germany).

The following landmarks were identified in the stained sections: the bottom of the bone defect (BD), and the coronal extension of the alveolar crest adjacent to the defect area (BC).

Defect length (mm) (DL) was measured from BC to BD, the width (mm) of the newly formed ridge (WR) was measured at 0%, 25%, 50%, 75%, and 100% of DL, the area (mm^2) of new bone fill (BF) was measured from BD to BC. Within BF, the area of newly formed mineralized (MT), and non-mineralized tissue (NMT), as well as the amount of remaining BOG, and BOB was calculated (mm^2) (Fig. 2).

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS 15.0, SPSS Inc., Chicago, IL, USA). Mean and SD values among animals were calculated for each variable and group. The data rows were examined with the Kolmogorow–Smirnow test for normal distribution. For the statistical evaluation of the changes within groups (i.e. changes over time), the paired t-test was used. For the comparisons between groups, the unpaired t-test was used. The α error was set at 0.05.

Results

The post-operative healing was considered as generally uneventful in all dogs. No complications such as allergic reactions, abscesses or infections were observed throughout the whole study period (Fig. 1f). However, after 3 weeks of healing,

all groups exhibited some augmented sites showing a pronounced hard tissue formation, identifiable as palpable exostoses. A premature exposure of the augmented sites was observed in one dog (8 weeks, LJ: BOG and BOG + rhBMP-2). Exposed sites were excluded from the statistical analysis.

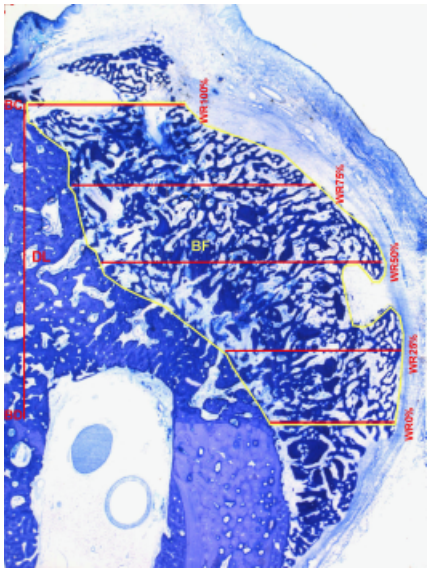

Fig. 2. The following landmarks were identified in the stained sections: the bottom of the bone defect (BD), and the coronal extension of the alveolar crest adjacent to the defect area (BC). Defect length (mm) (DL) was measured from BC to BD, the width (mm) of the newly formed ridge (WR) was measured at 0%, 25%, 50%, 75%, and 100% of DL, the area (mm^2) of new bone fill (BF) was measured from BD to BC. Within BF, the area of newly formed mineralized (MT), and non-mineralized tissue (NMT), as well as the amount of remaining BOG, and BOB was calculated (mm^2).

Histological observations/ histomorphometrical analysis

In general, at 3 and 8 weeks following bone grafting, all groups exhibited comparable mean values of DL in both UJ and LJ ($P > 0.05$, respectively; paired *t*-test) (Fig. 3).

At 3 weeks, histological observation revealed that BG ensured a homogeneous stabilization of BOG in the defect areas. Similarly, BOB still appeared to be fixated at both UJ and LJ in all groups. In general, histological observation failed to demonstrate obvious differences with respect to the pattern of bone regeneration as well as the organization of BOG and BOB scaffolds between groups (Figs 4 and 5). In particular, TB staining indicated that new trabeculae of woven bone mainly arised from open bone marrow spaces of the adjacent alveolar BD. The subsequently formed primary spongework of woven bone covered the defect area in coronal and lateral directions, primarily along and in close contact to the BOG and BOB scaffolds. In all groups, the peripheral part of the spongiosa appeared to be higher mineralized than in the centre. This was particularly true for BOB, showing a reduced ingrowth of trabecular bone within both biocoated and uncoated scaffolds (Figs 4 and 5). Immunohistochemical analysis revealed that the areas of newly formed bone were characterized by an intense OC staining of the mineralized matrix and the numerous osteocytes located in large lacunae. In all groups, NMT adjacent to both BOG and BOB revealed obvious signs of an OC antigen reactivity (Fig. 6).

At 8 weeks, wound healing was mainly characterized by an ongoing bone formation and mineralization. In particular, histological observation revealed a continuous filling of the intertrabecular spaces in all groups and subsequently a transformation into primary osteons. This was particularly true for the central parts of rhGDF-5, and rhBMP-2 biocoated BOB. However, a similar amount of bone resorption was identifiable in the peripheral compartments of all groups, resulting in an exposure of residual BOG and BOB scaffolds (Figs 7 and 8).

Mean WR values measured at 0%, 25%, 50%, 75%, and 100% of DL at both UJ and LJ in different groups are presented in Fig. 9. After 3 weeks of healing, all groups revealed comparable mean WR values in the LJ for BOG, as well as in the UJ for BOB ($P > 0.05$, respectively; paired *t*-test). However, significantly highest mean WR50 values were observed for rhBMP-2 biocoated BOG in the UJ, as well as BOB in the LJ ($P < 0.001$, respectively; paired *t*-test) (Fig. 9a). At 8 weeks, mean WR values tended to be lower in all groups, outlining that a remodelling of

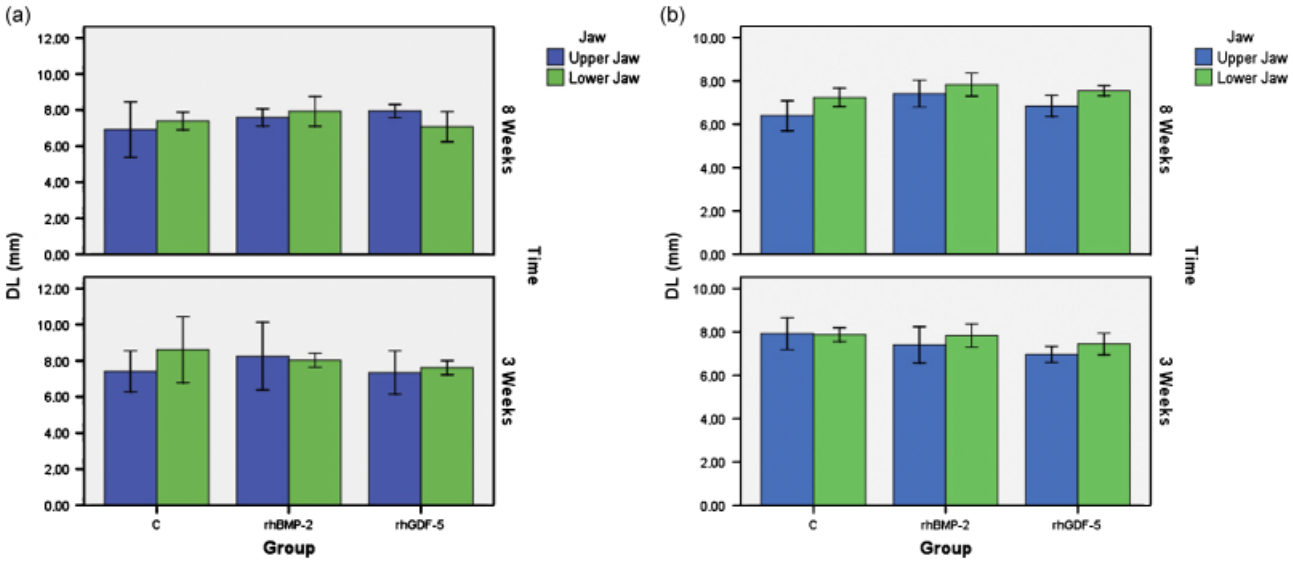

Fig. 3. Mean DL (mm ± SD) in different groups after 3 and 8 weeks of healing. (a) BOG (upper jaw: $n = 24$ sites; lower jaw: $n = 22$ sites). (b) BOB (upper jaw: $n = 24$ sites; lower jaw: $n = 24$ sites).

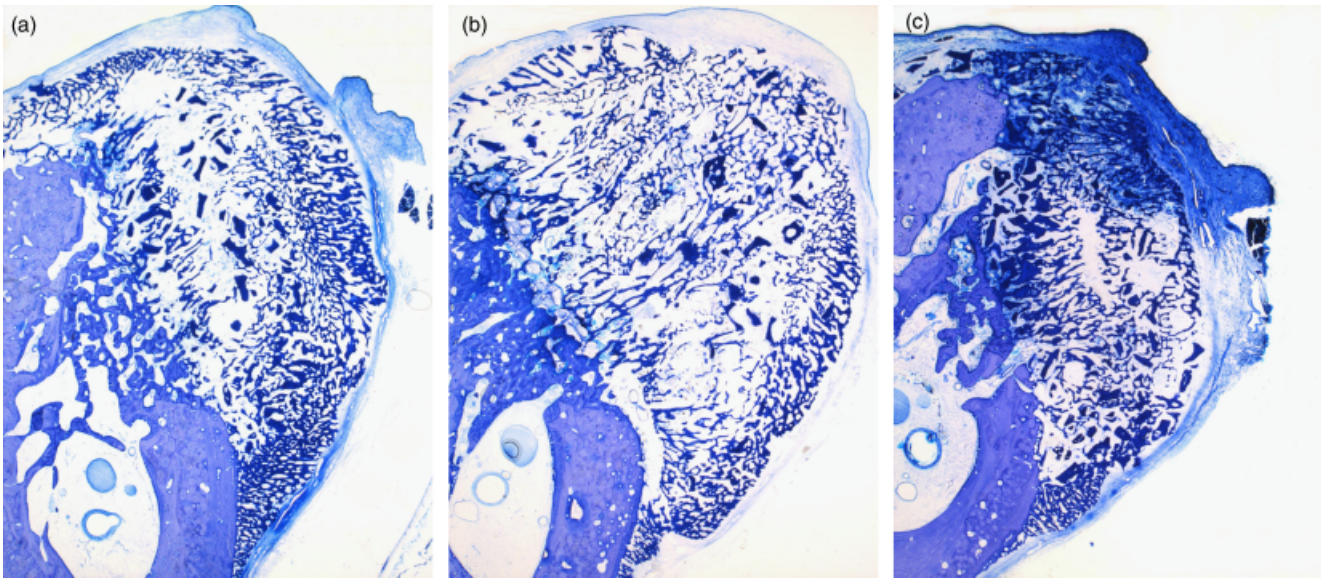

Fig. 4. Representative histological views of bone regeneration in the BOG groups after 3 weeks of healing (TB stain, original magnification ×12.5). TB staining indicated a similar pattern of bone regeneration in all groups. In particular, new trabeculae of woven invaded within and circumferentially around the BOG scaffold. (a) C (lower jaw). (b) rhBMP-2 (upper jaw). (c) rhGDF-5 (lower jaw).

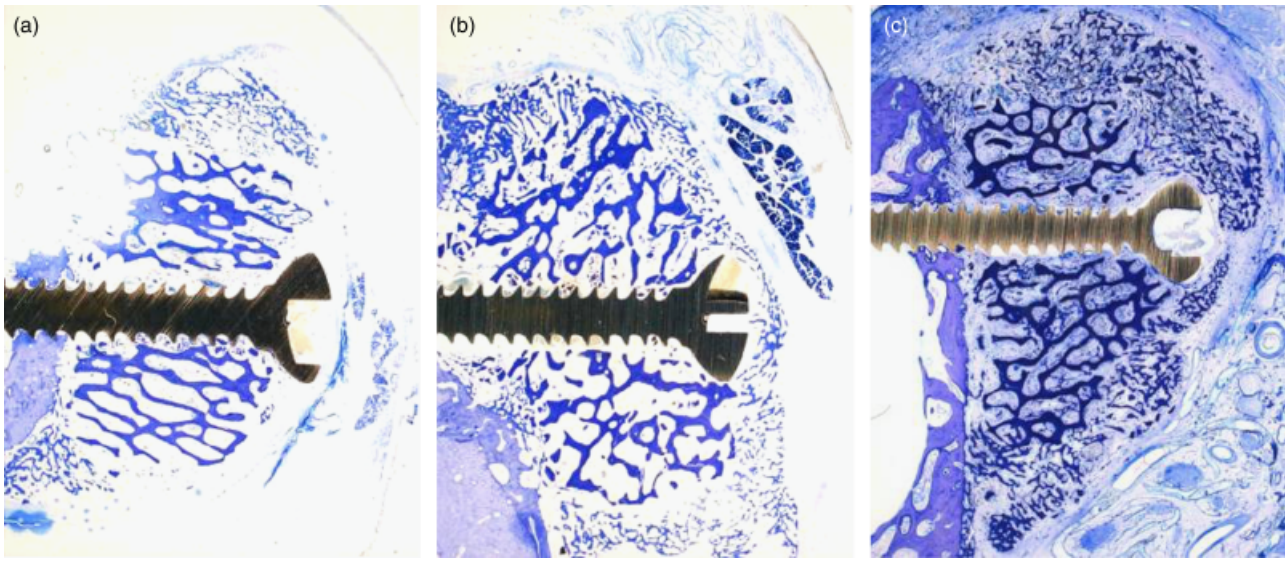

Fig. 5. Representative histological views of bone regeneration in the BOB groups after 3 weeks of healing (TB stain, upper jaw, original magnification ×12.5). The ingrowth of trabecular within the scaffold appeared to be reduced in all BOB groups. (a) C (upper jaw). (b) rhBMP-2 (upper jaw). (c) rhGDF-5 (upper jaw).

the newly formed bone had occurred between 3 and 8 weeks of healing. However, these differences did not reach statistical significance ($P>0.05$, respectively; unpaired *t*-test). While in general, mean WR values were comparable in all groups, statistical analysis revealed significantly highest mean WR20 and WR100 values for rhBMP-2 biocoated BOB and BOG in the LJ ($P<0.001$, respectively; paired *t*-test) (Fig. 9b).

Mean values of BF, MT, NMT, BOG, and BOB after 3 and 8 weeks of healing in all groups are presented in Fig. 10. In general, mean BOG and BOB values were comparable in all groups ($P>0.05$, respectively; paired *t*-test). At 3 weeks, rhBMP-2 biocoated BOG revealed significantly higher mean BF and MT values than the C group in both UJ and LJ ($P<0.01$, respectively; paired *t*-test) (Fig. 10a). While these values also tended to be highest for BOB, however, the differences did not reached statistical significance ($P>0.05$, respectively; paired *t*-test) (Fig. 10a). At 8 weeks, in the UJ, rhBMP-2 biocoated BOG revealed significantly higher mean BF and MT values than both rhGDF-5 and C groups ($P<0.01$, respectively; paired *t*-test). In both UJ and LJ of BOB, rhBMP-2 as well as rhGDF-5 revealed significantly higher MT values

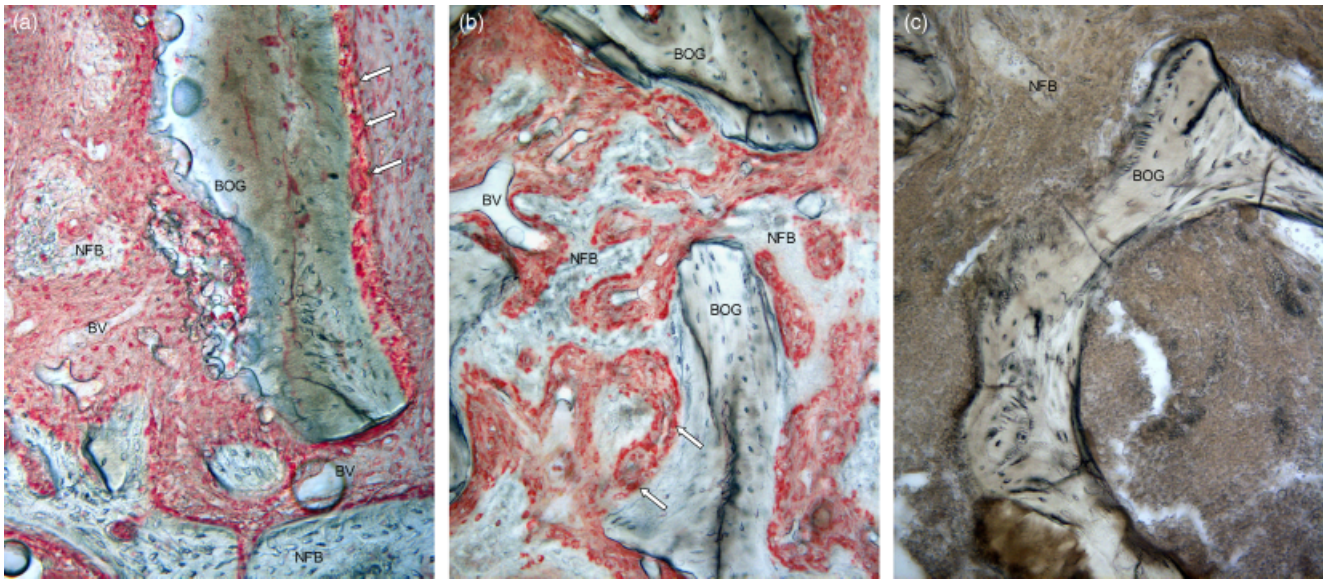

Fig. 6. Immunohistochemical analysis revealed obvious signs of an OC antigen reactivity (arrows) within the NMT adjacent to both BOG and BOB scaffolds of all groups. (a) BV, blood vessel; NFB, newly formed bone. (b) BOG/rhBMP-2 (lower jaw, original magnification × 200). (c) BOG/rhGDF-5 (lower jaw, original magnification ×200). (d) BOG negative control (lower jaw, original magnification ×200).

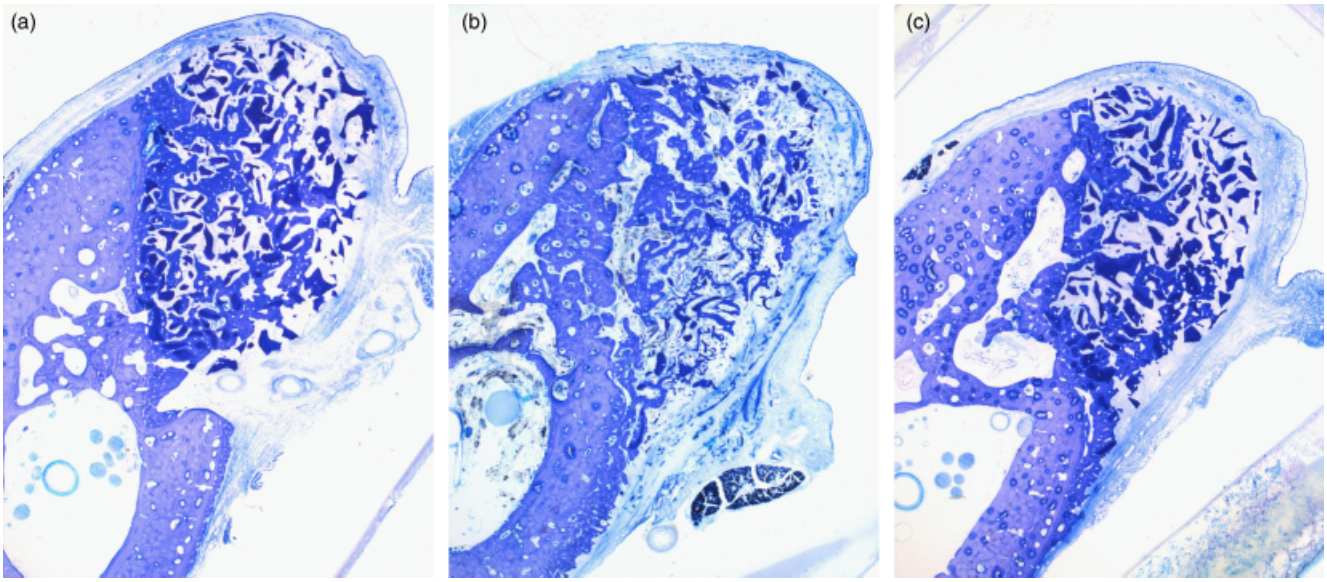

Fig. 7. Representative histological views of bone regeneration in the BOG groups after 8 weeks of healing (TB stain, original magnification ×12.5). In all groups, wound healing was mainly characterized by an ongoing bone formation and mineralization, resulting in a continuous filling of the intertrabecular spaces. This was particularly true for the central part of all augmented areas. (a) C (lower jaw). (b) rhBMP-2 (lower jaw). (c) rhGDF-5 (lower jaw).

than the C group ($P<0.01$, respectively; paired t-test) (Fig. 10b).

Discussion

The present study was designed to evaluate the influence of rhGDF-5 and rhBMP-2 biocoatings on lateral ridge augmentation using either BOG or BOB in a dog model. In general, it was observed that all treatment procedures resulted in considerable BF and MT values during the entire healing period of 8 weeks. This was particularly true for rhBMP-2 biocoated BOG, exhibiting significantly higher BF and MT values than the C group after 3 and 8 weeks, and even the rhGDF-5 group at 8 weeks in the UJ. While all BOG groups also exhibited a pronounced bone formation in the center of the grafted areas, the ingrowth of bone generally appeared to be lower for BOB. Even though rhBMP-2 biocoated BOB tended to show improved BF and MT values at 3 weeks, these differences did not reached statistical significance. However, after 8 weeks of healing, rhGDF-5 and rhBMP-2 biocoatings significantly improved the ingrowth of bone within the BOB scaffold, resulting in significantly higher MT values than in the C group. In all groups, immunohistochemical analysis revealed that NMT adjacent to BOG and

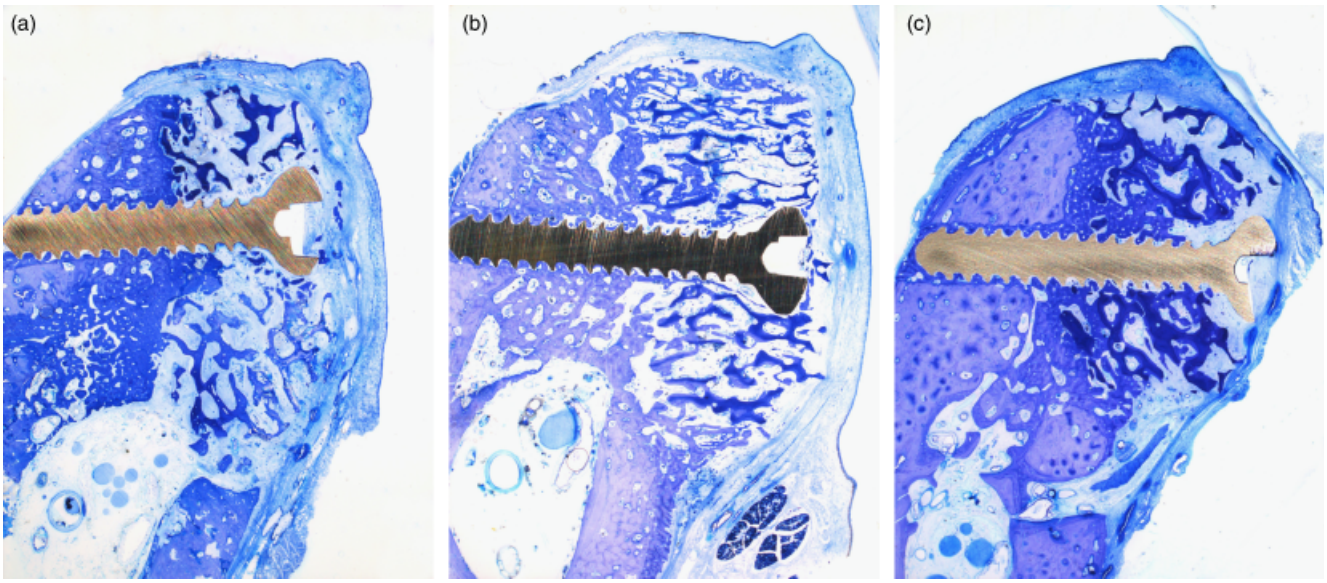

Fig. 8. Representative histological views of bone regeneration in the BOB groups after 8 weeks of healing (TB stain, original magnification ×12.5). The filling of the intertrabecular spaces was obviously pronounced at BOB biocoated with rhGDF5- and rhBMP-2. However, all groups revealed a certain amount of bone resorption in the peripheral compartment of the augmented areas, resulting in an exposure of residual BOG (see Fig. 7) and BOB scaffolds. (a) C (lower jaw). (b) rhBMP-2 (lower jaw). (c) rhGDF-5 (lower jaw).

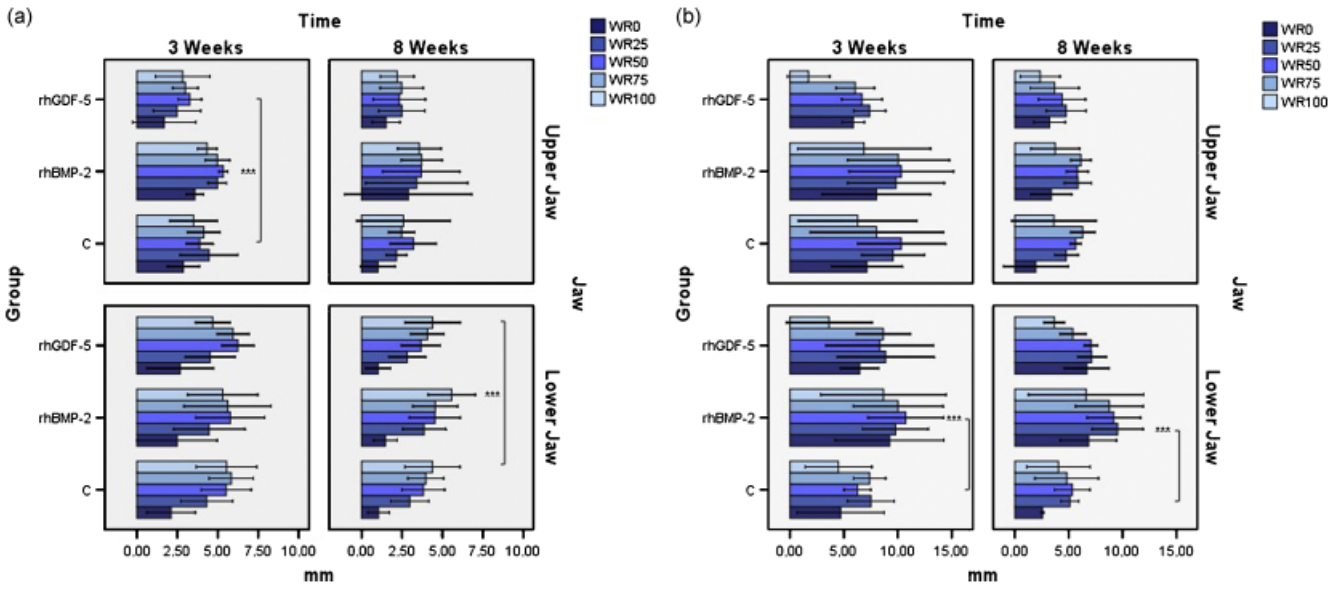

Fig. 9. Mean WR measured at 0%, 25%, 50%, 75%, and 100% of DL (mm ± SD) in different groups after 3 and 8 weeks of healing. (a) BOG (upper jaw: $n = 24$ sites; lower jaw: $n = 22$ sites). (b) BOB (upper jaw: $n = 24$ sites; lower jaw: $n = 24$ sites).

BOB was demarcated by a pronounced OC antigen reactivity. In this context, it must be pointed out that OC is one of the most abundant non-collageneous proteins of bone matrix, which is exclusively synthesized by osteoblasts, odontoblasts, and hypertrophic chondrocytes (Gallop et al. 1980). The present results noted for BOB alone are in agreement with previously published data (Araújo et al. 2002). In particular, at 6 months following lateral ridge augmentation in dogs, close to 30% of the height and 50% of the length of BOB was replaced with NMT, and only moderate amounts of new bone formation was observed particularly at the base of the scaffold. In this context, it must be pointed out that the design of the chronic-type lateral ridge defects was choosen according to the surgical procedure reported in previous studies employing the same model (von Arx et al. 2001a, 2001b; Araújo et al. 2002). To the best of our knowledge, these are the first histological data evaluating BOG for lateral ridge augmentation in dogs. However, the osteoconductive properties noted for BOG corroborate, to a certain extent, previous animal studies reporting on sinus floor augmentation, healing of self-contained BDs, or bone regeneration at dehiscence-type defects (Wetzel et al. 1995; Cho et al. 1998; Carmagnola et al. 2002). When interpreting

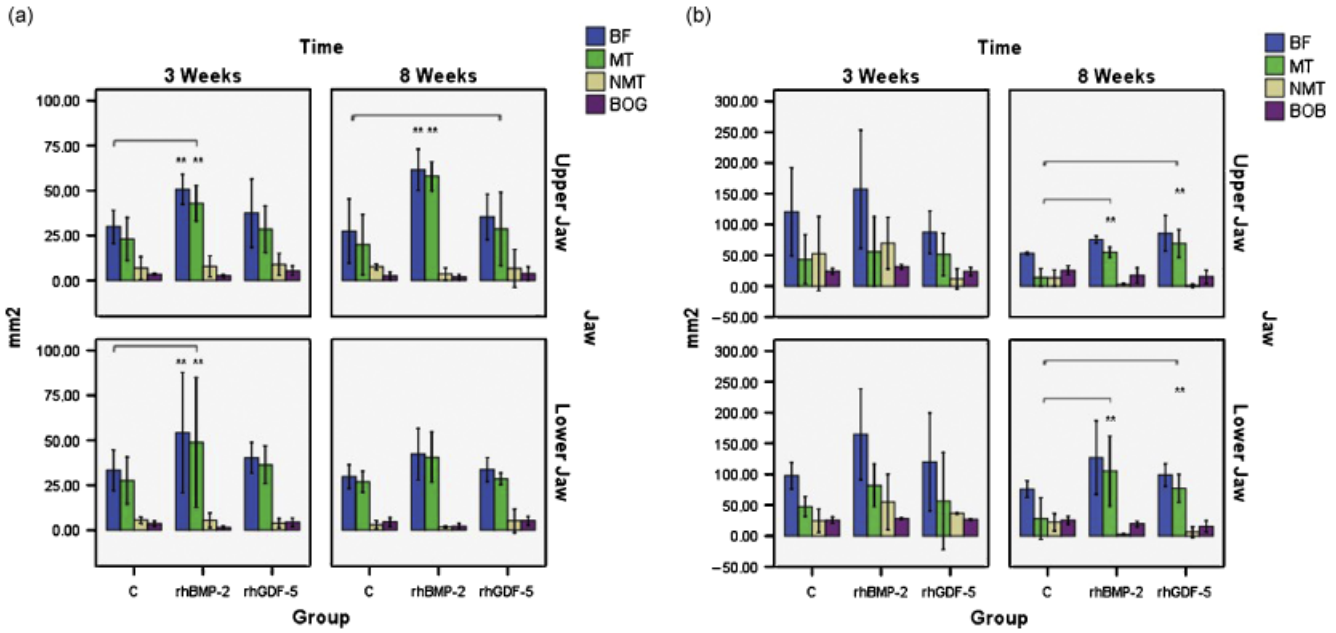

Fig. 10. Mean BF, MT, NMT, BOG, and BOB ($mm^2 \pm SD$) in different groups after 3 and 8 weeks of healing. (a) BOG (upper jaw: $n = 24$ sites; lower jaw: $n = 22$ sites). (b) BOB (upper jaw: $n = 24$ sites; lower jaw: $n = 24$ sites).

the present results, it was also observed that all grafted areas exhibited a certain amount of bone resorption between 3 and 8 weeks of healing. This remodelling was mainly limited to the newly formed peripheral bone surface, subsequently leading to an exposure of the BOG and BOB scaffolds in both groups. There might be several reasons to explain bone resorption after 3 and 8 weeks. First of all, it must be realized that a bone graft substitute that is placed on the lateral aspect of an edentulous ridge will stretch the adjacent mucoperiosteal flap, thus promoting osteoclastic activity in the augmented area. Since bone resorption was most pronounced at 8 weeks, it might be emphasized that BG was able to protect the augmented areas during initial stages of wound healing. Indeed, previous studies also reported on a certain amount of bone resorption following lateral ridge augmentation, particulary at non-protected sites (von Arx et al. 2001a, 2001b; Araújo et al. 2002). These observations might also be supported by the results of a recent study performed in dogs, indicating that a significant biodegradation of BG occurred bewteen 4 and 6 weeks of healing (Schwarz et al. 2007). On the other hand, however, some studies also provide evidence of delayed bone formation when rhBMP-2 was combined with barrier membranes made of expanded polytetrafluoroethylene (ePTFE) (Cochran et al. 1999; Jovanovic et al. 2007). This observation might be explained by the compromised vascularization of the wound area due to the occlusive characteristics of ePTFE membranes. In contrast, BG supported an early transmembraneous angiogenesis, thus providing cell resources essential to rhBMP-2 bone induction (Schwarz et al. 2007). Recently, the effects of rhBMP-2 biocoated BOG was also evaluated in a controlled clinical trial for guided bone regeneration using BG (Jung et al. 2003). While at 6 months, histomorphometrical analysis revealed a comparable mean area density of newly formed bone in both groups (test: $37 \pm 11.2\%$; control: $30 \pm 8.9\%$), the fraction of mineralized bone identified as mature lamellar bone was significantly higher at test ($76 \pm 14.4\%$) compared with control sites ($56 \pm 18.3\%$). This observation is in accordance with the present results, since rhBMP-2 biocoated BOG had a significant effect on the formation of MT after 3 and 8 weeks of healing. In this context, it must be emphasized that at 8 weeks, the beneficial effect of rhBMP-2 was restricted to the UJ. However, this was probably due to the number of prematurely exposed sites in the LJ, which had to be excluded from the statistical analysis. All these data, taken together with the results from the present study seem to indicate that rhBMP-2 might have a beneficial effect on the clinical outcome following lateral ridge augmentation using both BOG and BOB scaffolds. So far, rhBMP-2 was administered to saddle-type alveolar ridge, as well as supraalveolar periimplant defects mainly using absorbable collagen sponges (Sigurdsson et al. 1997; Tatakis et al. 2002; Jovanovic et al. 2007). While bone regeneration was comparable within a selected dose range of 0.05, 0.1, and 0.2 mg/ml (Tatakis et al. 2002), it appeared that a 0.4 mg/ml dose provided an enhanced bone induction (Sigurdsson et al. 1997). In the present study, rhBMP-2 biocoating of BDX and BOB was choosen according to the suggestions of Jung et al. (2003), even resulting in a two times higher dose of 0.77 mg/ml. In this context, it must also be emphasized that several *in vivo* studies have pointed to a limited dose response of rhBMP-2, once a certain treshold dose was reached (Sandhu et al. 1996; Wikesjö et al. 1999). However, due to the differences in carriers, defect models, and observations intervals employed in previous studies, it may be difficult to define an universal treshold dose for *in vivo* applications. Previous studies also investigated a dose dependency as well as the influence of different carrier materials on the bone regenerative effect of rhGDF-5 in a calvarial defect model (Kuniyasu et al. 2003; Poehling et al. 2006; Yoshimoto et al. 2006). In particluar, a dose response (1, 10, or 100 µg; 0.2, 2, or 20 µg) was

observed for a rhGDF-5/atellocollagen composite at 2 and 3 weeks after implantation (Kuniyasu et al. 2003; Yoshimoto et al. 2006). Similary, Poehling et al. (2006) reported on a five times higher new bone formation when rhGDF-5 was applied as MD05 (500 μg protein/g). At 6 weeks after implantation, defects filled with MD05 exhibited the highest bone density as well as a consistent and pronounced trabecular bone ingrowth. In contrast, wound healing in all other test (e.g. BOG) and control groups was mainly characterized by the formation of a loose fibroconnective tissue and only limited bone formation at the defect margins (Poehling et al. 2006). These observations are, to a certain extent, not in agreement with the results noted in the present study. In particular, after 3 and 8 weeks of healing, BOG exhibited excellent osteoconductive properties for bone ingrowth, resulting in mean BF, MT, and NMT values comparable with that noted for rhGDF-5 biocoated BOG. In this context, it must also be realized that rhGDF-5 was even used at a higher concentration of 768 μg protein/g. There might be several reasons to explain this discrepancy. First of all, it must be emphasized that potential differences might be related to the animal species as well as the defect model investigated. Secondly, the rat calvarial defects were not covered by a barrier membrane, thus allowing the premature ingrowth of connective tissue. Finally, different release characteristics of either β-TCP or BOG might also have influenced the effectiveness of rhGDF-5 in both studies.

Within the limits of the present study, it was concluded that both rhGDF-5 and rhBMP-2 have shown efficacy, however, their bone regenerative effect was markedly influenced by the carrier.

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