Impact of rhBMP-2 on regeneration of buccal alveolar defects during the osseointegration of transgingival inserted implants

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Objective. New approaches to enhance vertical bone regeneration in clinically relevant implant models are needed. Therefore, we analyzed the impact of recombinant human bone morphogenic protein 2 (rhBMP-2) on the healing of large buccal alveolar defects during osseointegration of transgingivally inserted implants.

Study design. Twenty-four dental implants were inserted transgingivally in the mandibles of 6 labrador/golden retriever cross-bred dogs. Before implantation, a standardized buccal bone defect was created and refilled with either calcium phosphate as a carrier containing rhBMP-2 or calcium phosphate alone. Either ceramic abutments that enabled immediate implant loading or healing distance collars to prevent loading were mounted. Sixteen weeks after intervention, bone implant units were analyzed by radiofrequency analysis and histomorphometry.

Results. In total, 14 implants (58.3%) were available for further analysis. The mean depth of the bone defects, the gain of regenerated bone, the vertical osseointegration of the implants, and the bone-to-implant contact in the newly formed bone were slightly greater in the rhBMP-2–containing samples. In contrast, the osseointegration in the preexisting bone was even superior within the non-rhBMP-2–treated specimen. However no differences were statistically significant.

Conclusions. When rhBMP-2–conducted bone regeneration was compared with control samples, no significant differences of newly formed bone were found at the bone-implant interface. The amounts of rhBMP-2 applied do not seem suitable to enhance implant osseointegration in large buccal defects. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;xx:xxx)**

There is a therapeutic need to stimulate new bone growth in implant dentistry owing to the fact that the latter is gaining popularity in an aging population. Owing to naturally occurring ridge resorption following tooth loss, implant sites often lack sufficient bone for dental implantation. Therapeutic approaches to achieve implant-guided bone growth using an osteogenic agent can principally overcome these disadvantages. Bone morphogenetic proteins (BMPs), a subgroup of the transforming growth factor β superfamily, were discovered based on a bone-inductive activity concealed in bone matrix.[1] Recent studies have shown that BMP is present in the matrix of dentin,[2-4] bone,[5,6] heterotopic bone formation, and lesions after tooth extraction.[7,8] BMPs play 2 essential roles within the muscular and skeletal system and the tissue differentiation during embryonic development. The most important role of BMPs is to regulate the key elements in the bone induction cascade required for regeneration of skeletal tissues, as described by Reddi,[9,10] Bostrom and Asnis,[11] Barnes et al.,[12] Bessa et al.,[13,14] and Smith et al.[15]

Recently, it has been shown in several studies that recombinant human BMP-2 (rhBMP-2) has an explicit beneficial effect on establishing and improving the contact between an implant and its surrounding bone.[16-18]

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Clinically, rhBMP-2 has induced relevant bone formation in a multitude of craniofacial and periodontal settings in large animal models[19-22] as well as in, e.g., fracture healing of rabbits.[23] Furthermore, evidence exists that the use of carriers which release osteoinductive substances over a certain amount of time is beneficial to the regeneration of the bone implant interface. However, it is still a matter of controversy whether or not carriers are beneficial for the delivery of BMP. Drug delivery systems, in the form of implant coatings or scaffolds placed around implants, must be used for sustained and controlled release of rhBMP-2, because it has been shown in vivo to diffuse rapidly away from the implant site.[24] Lindholm and Gao[25] and Boden[26] considered that growth factors can exert their biologic activity optimally only in combination with a carrier. In contrast, other authors considered that a carrier is biologically not mandatory for the effective use of a growth factor.[27] However, BMPs act locally and therefore must be delivered directly to the site of regeneration via a carrier.[28] Sakou[29] showed that the use of a carrier is beneficial in increasing the induction of newly formed bone and in improving the reproducibility of the results. It has been proven that BMPs have osteoinductive effects in combination with both organic and anorganic carriers.[30-33] Importantly, the following characteristics can be postulated for carrier materials: binding the active protein, prevention of unspecific proteolysis, biocompatibility and biodegradability, maintaining a local biological effective concentration of BMP, and no interference with the wound-healing process. Frequently used carriers that promote the induction of bone formation by BMP are hydroxyapatite,[34] demineralized bone matrix,[35] high-molecular-weight compounds,[36] and collagen.[37] However, currently no optimal carrier material exists that is at the same time optimally biodegradable and dimensionally stable. It has recently been shown that a successful bone regeneration after sinus floor elevation and in extraction alveolus could be achieved by the application of collagen sponges that were impregnated with rhBMP-2.[38-40]

Nevertheless, collagen is an allogenic material associated with the risk of transmitting slow virus diseases. In addition, incorporated BMP molecules are still released rapidly in a single-burst fashion, and the resulting high local protein levels thereby result in undesirable nonspecific binding to collagen fibrils and other extracellular matrix molecules in the vicinity of the implant.[16] Therefore, to circumvent this difficulty, other, more natural, carriers such as calcium phosphate (CP) have been introduced. Preformed CP layers have also been chemically modified in an attempt to delay the release of adsorbed growth factors and to restrict the osteoinductive effects of incorporated bioactive agents temporospatially.

To overcome undesirable effects of high amounts of BMP-2 and for cost reasons we reduced the effective dose of BMP-2 down to 2 μg/mL carrier compared with earlier experiment.[16]

Owing to the fact that the vast majority of studies have analyzed the effect of rhBMP-2 on unloaded closed-healing implant situations, we created the study being aware of the much more difficult circumstances of transgingivally inserted implants with immediate loading. The aim of the present study was to analyze the effect of CP-bound rhBMP-2 on bone regeneration around transgingivally inserted oral implants into a region with a severe buccal bone and periosteal defect. In addition, we analyzed if rhBMP-2 can still be effective without the periosteum as a highly potent source of bone regeneration.

Furthermore, the hypothesis should be tested whether rhBMP-2 released out of the CP carrier increases the quality and structure of the local bone implant contact (BIC) in a transgingivally insertion model with immediate and unloaded loading. Untreated buccal defects around implants served as control.

MATERIALS AND METHODS

Animals and materials

Six full-grown male labrador/golden retriever crossbred dogs aged 13-20 months and weighing 25-30 kg were included in this study. The study was performed according to the approval of the Institutional Animal Care Committee of the University of Aachen, Germany.

Two weeks before surgery, dental calculus and soft films on the teeth were entirely removed under intravenous anesthesia with acepromazinmaleate (0.02 mg/kg; Albrecht, Aulendorf, Germany), levomethadonhydrochloride (0.2 mL/kg; Hoechst Roussel Vet, Unterschleissheim, Germany), and entobarbital sodium (0.2 mL/kg; Rhone Merieux, Laupheim, Germany) to reach clean conditions of the parodontium and teeth before the operation. To reduce the total number of animals needed, multiple implant sites were investigated within the same dog.

In each experimental series, 4 Ti-Unite dental implants with a diameter of 3.75 mm and a length of 10 mm (Brånemark System Replace, Nobel Biocare, Gothenburg, Sweden) were inserted according to the original standard protocol of the manufacturer.

In every operational section, general anesthesia was performed by the following guidelines as subcutaneous administration of the broadband antibiotic enrofloxacin (2.5 mg/kg; Bayer Vital & Co., Leverkusen, Germany) and the analgesic caprofen (Pfizer Animal Health, Karlsruhe, Germany). The animals were sedated by

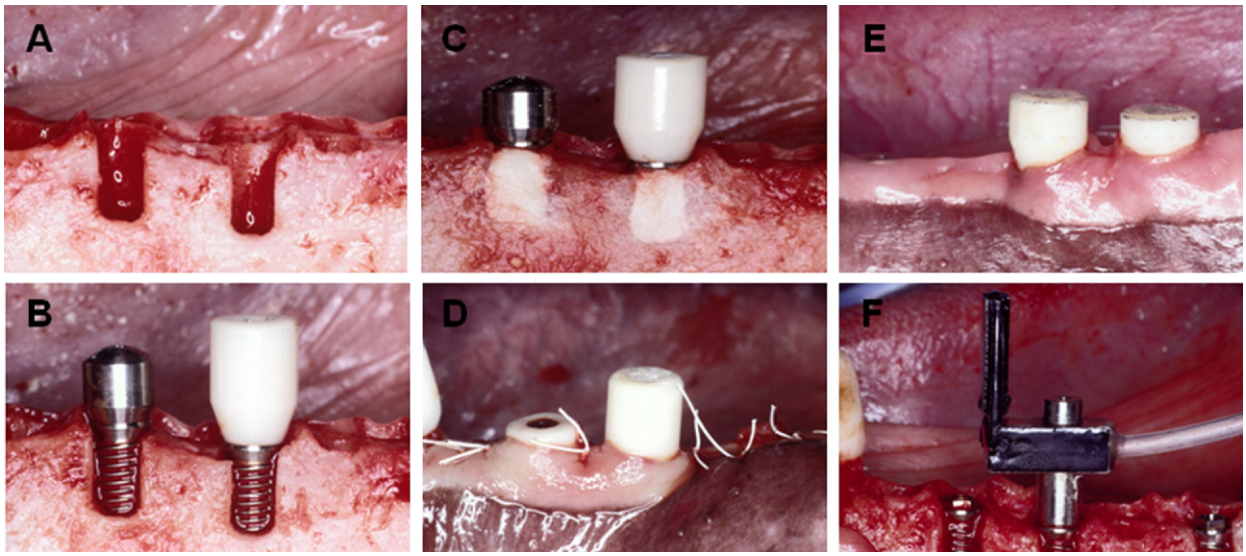

Fig. 1. Dog's jaw with buccal bone defects (**A**) and after insertion of implants (**B**). **C,** Padding of the buccal defects with the carrier loaded with and without rhBMP-2. **D,** Fixation of the mucoperiosteal flap around the distance collars with horizontal mattress sutures and single stitches (view from buccal side). **E,** Gingival tissue 5 weeks after implant healing. **F,** Resonance frequency analysis to determine the stability of the implants.

intravenous administration of levomethadonhydrochloride (0.2 mL/kg), intubated, and anesthetized with a mixture of oxygen (24%), halothane (75%), and isoflurane (1%) and with pentobarbital sodium (0.2 mL/kg; Rhone Merieux). Additionally, conduction and infiltration of local anesthesia at the operation site was performed by xylocaine 2% with adjuvant of 1:50,000 epinephrine (Astra Zeneca, Zug, Switzerland).

In the first operational section, all 4 mandibular molar teeth were extracted via an intrasulcular incision starting from the canine up to the second molar followed by reflection of a conventional mucoperiosteal flap. The wounds were then closed by single sutures (Fig. 1, *C* and *D*). Over the first 14 days, the dogs received soft diet, and after a healing period of 3 months, 1 lower quadrant of each dog was selected at random and a clear defined alveolar crest incision with buccal vertical cuts at each end to relief tissue stress was performed. The mucoperiosteal flap was dissected, and 2 drilling holes were carried out to prepare the insertion of 1 Brånemark dental implant (3.75 × 10 mm; Nobel Biocare, Goteborg, Sweden) according to the manufacturer's instructions (Fig. 1, *A-E*). Before the insertion of the implants, 2 drilling holes had been chosen at random, and reproducible buccal dehiscence defects, including periosteal resection of an area of 3 × 6 mm each, had been created in a standardized procedure (Fig. 1, *A*). After the insertion of transmucosal healing abutments and equivalent ceramic abutments, the mucoperiosteal tissue was carefully readapted to the distance collars and fixed with horizontal mattress- and single-stitch sutures (Gore-Tex suture CV5; W. L. Gore and Associates, Flagstaff, AZ). To assure a transgingival implant healing process with osseointegration, ceramic abutments were screwed onto the implants in the regions of the right and left second premolars, and healing distance collars onto the implants in the regions of the right and left first molars. A total of 24 dental implants were inserted. Out of these, 12 implants were immediately exposed to mastication by application of a ceramic abutment (Fig. 5). The remaining 12 implants were exempted from loading during the healing period by titanium or ceramic distance collars screwed on transgingivally. On one side of each mandible, the previously created buccal bone defect was filled up with rhBMP-2, incorporated and adsorbed into biomimetic CP [150 μL rhBMP-2–CP each with an effective concentration of 2 μg/mL carrier, at 37°C, resulting in 0.3 μg per buccal defect; The CP carrier was developed and delivered by the Department of Physiologic Chemistry, University of Duisburg-Essen. The rhBMP-2/ACS [InductOs] was received from Wyeth USA. On the contralateral sides, which served as control, the bone defects were filled with native CP only.

Initially and 4 months after implant placement, osseointegration of the remaining implants was analyzed by radiofrequency analysis[41] and all bone-implant units were finally embedded for histomorphometry (Fig. 4).

Histology

The mandible of each killed animal was removed in its entirety after a consolidation period of 16 weeks. The molar regions were divided into 4 parts (right and left second premolar and first molar regions) of implantation and tissue augmentation, each including 1 implant and the surrounding bone and soft tissues.

The specimens were prepared according to the technique of Donath.[42] In brief, the tissue samples were cut through the midaxial line of the implants in buccolingual orientation into slices of 200 μm by using a diamond-coated band saw. Three consecutive cuts were performed of each specimen and marked as cuts 1, 2, and 3. Each cut was then trimmed to a final thickness of 20-30 μm by using an Exakt grinding machine (Exakt, Norderstedt, Germany) and stained with toluidine blue. The nondemineralized tissue cuts were analyzed histometrically under light microscopy at ×250 magnification by using a computer-assisted imaging system (Image-Pro Plus; Leica, Mikrosysteme Vertriebs, Bensheim, Germany).

The following histomorphometric parameters were measured in the buccal and lingual dimension, the examiner was blinded to the previous implant position and the respective therapy group (Fig. 3):

1. Depth of the bone defect: distance (mm) between the reduced alveolar crest of the alveole (bottom point of the defect) and the shoulder of the implant along the implant surface.
2. Vertical gain of bone: distance (mm) between the reduced alveolar crest (bottom point of the defect) and the most coronal point of the new bone.
3. Vertical osseointegration: distance (mm) between the most coronal point of contact of the regenerated bone and the thread of the implant and the bottom point of the defect measured in the coronal direction.
4. Bone-to-implant (BIC) contact new bone: relative BIC surface (%) at the area of the regenerated bone.
5. BIC preexisting bone: relative BIC surface (%) at the area of the preexisting bone.

Statistical analysis

To determine differences between the rhBMP-2–guided bone regeneration and control sites on the osseointegration of the implants, the Student *t* test for unpaired samples was used; to compare immediate loading of the implants in the premolar region with the unloaded molar implants on the respective side of the jaw, data were calculated with the Student *t* test for paired samples. Statistical significance was set at $P < .05$. The statistical analysis was performed using SPSS for Windows software (Version 14.0; SPSS, Chicago, IL).

RESULTS

Clinical results

Only 14 implants (58.3%) were available for further analysis owing to the effect of different mastication type in dogs with more transversal forces acting on the implants in the initial healing period even under a strictly soft diet. No visible signs of infection occurred at the 14 osseointegrated implants. Directly after implantation, resonance frequency analysis measurements (Fig. 1, *F*) were performed and reached values of at least 6,000 Hz to prove primary sufficient stability of the inserted implants after insertion with 30 N·m torque.[41]

No certain pattern of implant failure was evident. When considering the lower jaw, on 1 occasion failure of an implant in the region of the second premolar of the right side and in 4 occasions failure on the left side was observed. In the region of the first molar implant, failure was seen twice on the right side and 3 times on the left side: Animal no. 1 lost both molar implants, no. 3 lost the second premolar implant on the left, nos. 2 and 4 came out without any implant loss, no. 5 lost all but the first molar implant on the right side, and no. 6 lost all implants.

Sixteen weeks after implantation, neither residuals of the CP carrier nor signs of foreign body reaction could be detected in the histologic examinations. Histologically, both kinds of bone defects (treated with or without rhBMP-2) of the transgingivally healed implants showed only very little bone gain. Metrically, there was no difference between the study group and the control (Fig. 2).

Differences between newly formed bone and preexisting alveolar crest were apparent after staining of the thin-section slides with toluidine blue. Trabeculae were surrounded by osteoblast-like cells. There were also deposits of osteoid in the immediate vicinity (Fig. 3). Another remarkable finding was the extent of horizontal bone regeneration in the buccolingual direction within the bone defects, regardless or whether or not they were treated with rhBMP-2. Owing to the complexity of the created defect, bone regeneration required was not only in the vertical but also in the buccolingual direction. Therefore, it did not reach the extent of the original alveolar crest in the present study.

Quantitative histomorphometric analysis

The depth of the bone defects was measured histomorphometrically as described above. For the implants of the rhBMP-2 group there was a median depth of 5.72 mm (±0.48 mm). For the implants of the control group there was a median depth of 5.51 mm (±0.84 mm). There were no significant differences between the study and control groups ($P = .859$; Fig. 2, *A*). Within the

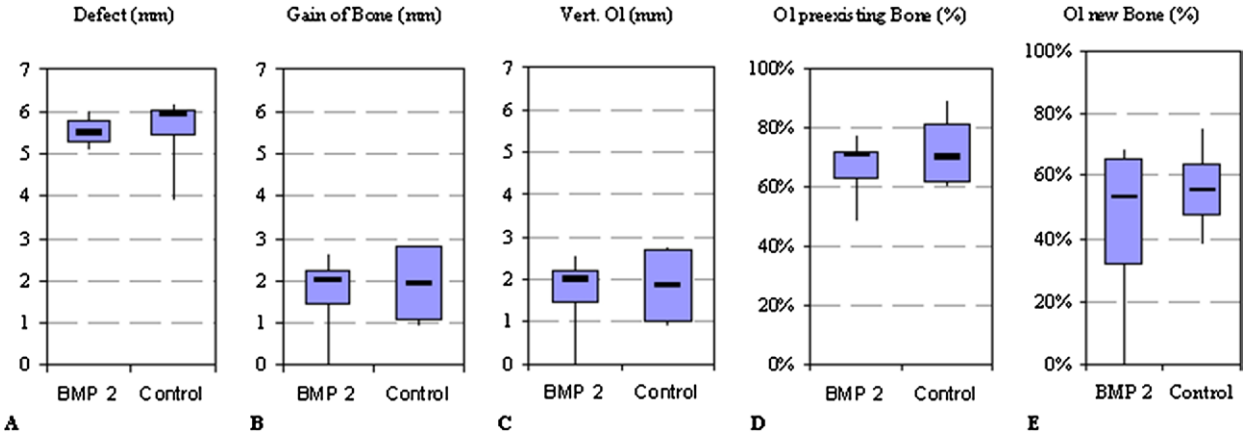

Fig. 2. Boxplot overviews of the results. Depth of the bone defect (**A**), vertical gain of bone (**B**), extent of vertical osseointegration (**C**), bone-to-implant contact newly formed bone (**D**), and bone-to-implant contact preexisting alveolar crest bone (**E**). *OI*, Osseointegration.

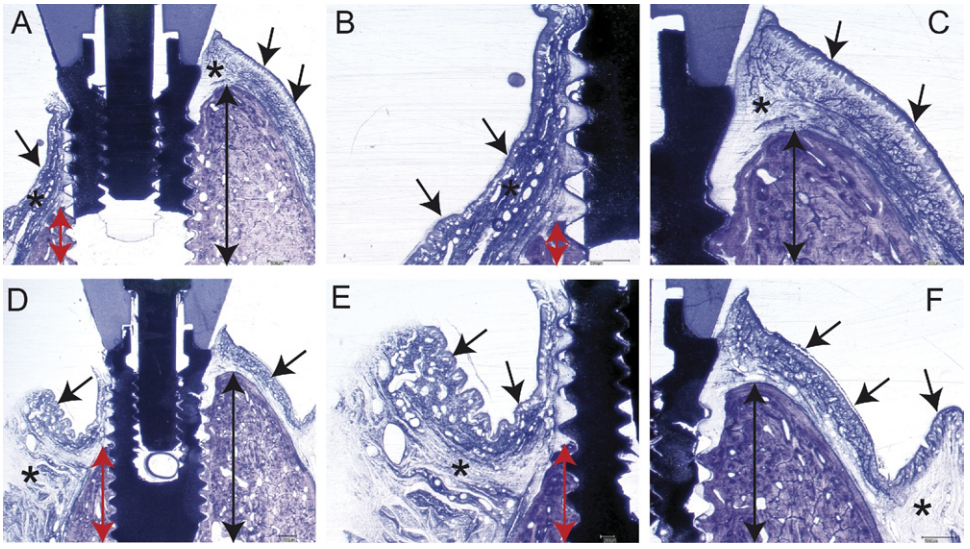

Fig. 3. Representative histology of the implant situation in regions 35 (**A-C**) and 45 (**D-F**). Buccal bone gain (new bone is marked by *red double arrows*). The regenerated bone is surrounded by connective tissue (*asterisks*) and covered by gingiva (*arrows*) as at the local bone on the opposite side (*black double arrows*). Section slide, toluidine blue. **A, D,** Overview (×40 magnification); **B, C, E, F,** detail views (×100 enlargement).

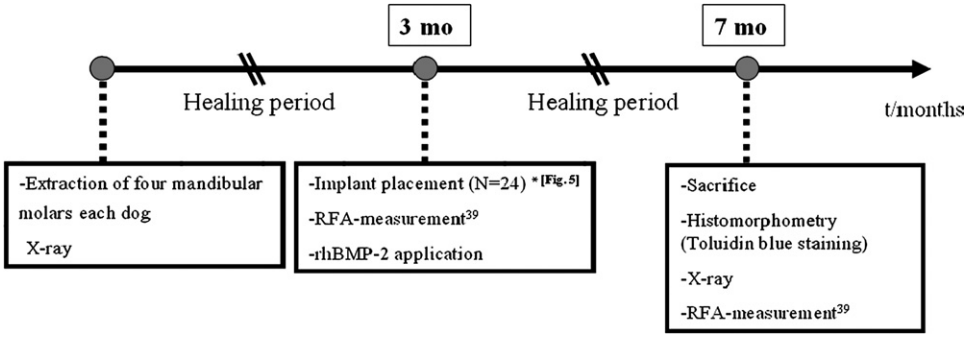

Fig. 4. Time schedule of experiment. *RFA*, Radiofrequency analysis.[41]

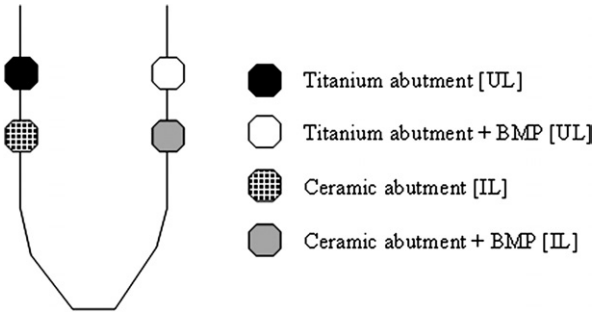

Fig. 5. Distribution of different implants and loading conditions within one animal. *UL*, Unloaded; *IL*, immediate loading.

Table I. Summary data of rhBMP-2 versus control

	rhBMP-2	*non-rhBMP-2*
Depth of bone defect (mm)	5.72 ± 0.48	5.51 ± 0.84
Vertical gain of bone (mm)	2.37 ± 0.66	2.25 ± 0.67
Vertical osseointegration (mm)	2.37 ± 0.63	2.2 ± 0.64
Bone-to-implant contact new bone (%)	66.5 ± 20.91	62.5 ± 12.05
Bone-to-implant contact preexisting bone (%)	67.17 ± 7.17	70.2 ± 40.95

group treated with rhBMP-2, the vertical bone gain was more important. The implants treated with rhBMP-2 showed a mean bone growth of 2.37 mm (±0.6 mm), whereas the mean bone growth of the implants without rhBMP-2 was 2.25 mm (±0.67 mm). These data also showed no significant differences between the groups ($P = .688$; Fig. 2, *B*). The integration of bone in the vertical direction was higher in the group treated with rhBMP-2 than in the control group. The mean amount of bone formation for implants treated with rhBMP-2 was 2.37 mm (±0.63 mm). For implants without rhBMP-2, the mean amount of bone was 2.2 mm (±0.64 mm). Regarding bone regeneration in the vertical direction, there were no significant differences between the study group and the control group ($P = .861$; Fig. 2, *C*). Implants treated with rhBMP-2 showed a higher total amount of bone within the regenerated bone mass. They showed a bone growth of 66.5% (±20.9%). Control implants demonstrated a mean value of 62.5% (±12.1%). These differences between the groups also were not significant ($P = .291$; Fig. 2, *D* and Table I). The amount of bone within the preexisting alveolar crest was even higher in the control group not treated with rhBMP-2 [70.2% (±15.0%)] compared with the quantity in the rhBMP-2 group [67.2% (±7.2%)]. Similarly to the other histomorphometric parameters, these differences were not significant ($P = .528$; Fig. 2, *E*). The complete histomorphometric data of the comparison of BMP-2 versus control are summarized in Table I.

Table II. Summary data of immediate loading (IL) versus unloaded (UL)

	IL	*UL*	*P value*
Depth of bone defect (mm)	5.47 ± 0.33	5.72 ± 0.66	.495
Vertical gain of bone (mm)	1.66 ± 0.7	1.9 ± 0.9	.833
Vertical osseointegration (mm)	2.2 ± 0.35	2.22 ± 0.51	.967
Bone-to-implant contact new bone (%)	58 ± 21.4	61.83 ± 17.17	.693
Bone-to-implant contact preexisting bone (%)	68.33 ± 10.41	70.11 ± 13.11	.774

When comparing the immediately loaded premolar implants with the unloaded molar implants, all histomorphometric parameters also revealed no significant differences. These data re summarized in Table II. There was not even a trend toward better results for the unloaded implants.

DISCUSSION

In the last few years it has become a trend to use a 1-stage procedure for implantation after dental loss.[43] In such situations, or in settings where the implant has to be put into an alveolus showing healing gingival tissue, a good surface-to-bone contact by building additional newly formed bone is mandatory. To meet this need, there have been many different approaches to promote the building of new bone mass by using osteogenic growth factors during the implantation procedure. The aim of the present study was to examine the effect of rhBMP-2 on bone regeneration in areas of severe bone defects at the healing sites of dental implants in a large animal model. The BMP-2 carrier potential of different materials has been tested at both ectopic and orthotopic sites.[44-46] Owing to the fact that in all cases the adsorbed agent was liberated too rapidly to induce a sustained osteogenic response, in the present study CP was chosen as a natural carrier and delivery system for rhBMP-2. Because CP is a natural organic part of the bone it proved to be advantageous in previous studies.[47,48] A special biomimetic technique, described previously, was used to integrate rhBMP-2 into the CP carrier to overcome the problem of unnatural inorganic coating-techniques as plasma spraying, sputtering or electrophoresis.[49] With this biomimetic technology,[49-53] rhBMP-2 can be incorporated into the 3-dimensional crystal latticework, from which it is released gradually in vivo when undergoing degradation. In a recent study by Liu et al.,[54] BMP-2 was incorporated into biomimetic CP coatings to induce ectopic bone formation. Resorbable CP-based cements have received

regulatory clinical approval for different defect repairs.[55] Calcium phosphate was chosen as the carrier for rhBMP-2 because it appropriately retains therapeutic doses of BMP at the defect site for a sufficient amount of time in the sense of temporospatial control and to stimulate target cells and induce the bone repair. Unique features of this cement are that it sets endothermically, is easily injectable through a 16-18-gauge needle, and is remodeled like normal bone through cell-mediated action.[56,57] In addition, this carrier exploits the natural high affinity of rhBMP-2 for CP materials, such as bone.[51] The therapeutic dose may vary between different animal and bone defect models. For the present model, previously published studies indicated a 2-week rhBMP-2 residence time as sufficient to enhance healing.[16,58] Compared with the study by Hanisch et al.,[16] the amount of BMP-2 incorporated into the carrier was much lower to prevent undesired effects of BMP-2, such as ectopic bone formation, and to test a dosage which is cost-effective for the long-term goal of routine human application.

Despite the superior coating technology for physiologic sustained release of rhBMP-2, the results of the present study showed no significant differences in the amount of newly formed bone mass 16 weeks after implantation between the study group treated with rhBMP-2 and the control group. In addition, there was no difference between unloaded and immediately loaded implants in this experiment. Improvement of implant healing by using natural BMP has been demonstrated in many experimental studies and patient surveys.[59-62] Later, these results could be confirmed for rhBMP.[63] Very high reproducible BIC ratios of up to 80% could be observed by other groups.[64] The main goal of the present study was to establish transgingival implant healing with possible bone regeneration in the presence of a severe buccal bone defect, simulating a compromised periodontal situation. No significant differences in vertical gain of bone and BIC could be observed between the bony defects treated with rhBMP-2 and controls after 16 weeks. There were only slight trends of lesser depth of the bone defects, higher vertical bone gain, and a higher vertical osseointegration within the group treated with rhBMP-2. We found no significant differences in new bone formation or in the percentage of BIC between the groups treated and not treated with rhBMP-2 after 16 weeks. These unexpected results may be explained by several factors of the study design. It is well known that there is a dose dependency for the effect of rhBMP-2.[65] Therefore, to obtain further bone formation, larger amounts of the recombinant growth factor are needed. In contrast to the 0.3 μg per implant defect applied in the present study, other experiments with rhBMP-2 were conducted with higher levels of BMP, up to the milligram range. Interestingly, we saw a relatively high SD of the BIC in the BMP-2 group compared with control. A wide interanimal variation of the osteogenic effectivity is described, which supports our BIC data.[17]

A similar study by Jones et al.[66] confirms the result of the present study. In Jones et al.'s study, the 4 lower premolars and the first molars of 12 dogs were extracted. Five months later, 2 implants were inserted on each side. A standardized bone defect of 4 mm was created around the implants. The rhBMP-2 was put into the bone defects of 1 side with a collagen carrier and a poly(lactic-*co*-glycolic acid) (PLGA) carrier. The control side was treated with the native carrier only. Half of the implants were covered with a polytetrafluoroethylene (PTFE) membrane to prevent soft tissue invasion. The results were measured at 4 and 12 weeks after implantation. After 4 weeks, a significant difference in BIC was observed between the group treated with rhBMP-2 and the control side, whereas 8 weeks later, with progressive healing, no significant differences between the 2 groups could be found. There was also a significant difference with increased bone gain in the group treated with a collagen carrier. The treatment with PTFE membranes delayed the building of new bone after 4 weeks. Stadlinger et al.[67] obtained similar data by application of the relatively low dosage of 400 ng rhBMP-4 in a collagen/chondroitin sulfate carrier with almost no benefit for the growth factor only group. Bax et al.[23] also observed a similar effect of rhBMP-2 on tibial fractures in rats, where the total amount of newly formed bone was not higher in the growth factor group than in the control. In the initial stage of the study only, rhBMP-2 accelerated the rate of callus formation.

Ishikawa et al.[68] examined the periodontal regeneration on artificially constructed bone defects with 3 walls in dogs with an rhBMP-2 concentration of 2 mg/mL and PLGA particles as carriers. Compared with the control, bone defects which remained entirely untreated and were only covered by mucoperiosteal flaps, the bone defects treated with rhBMP-2/PGLA showed considerably more bone and cement regeneration as well as formation of connective tissue–like attachment.

Sciadini et al.[69] used an absorbable collagen sponge (ACS; type I bovine collagen) as a carrier in their study of segmental bone defects of the radius of dogs. The control group was treated with autologous bone without rhBMP-2. This study also used different concentrations of rhBMP-2 (up to 0.8 mg/mL implant volume. The radiologic examinations showed that the implant treated with rhBMP-2/ACS had completely healed up after 12 weeks. The biomechanical properties of the regenerated bone showed equal or slightly better results for the side

with the implant versus the control side. There was an inverse dose dependency on the concentration of the growth factor. The lowest concentration of rhBMP-2 of 0.05 mg/mL delivered the best results for bone remodeling and biomechanical bone properties.[69]

The results of the present study should be considered regarding the lower bone regeneration and that the chosen buccal bone defect including periosteal resection constituted a complex periodontal defect, which can often be found in an elder patient before implantation. Even in patients without periodontal defects, smaller buccal lesions only heal with difficulty.[70]

Additional application of BMP-2 at the side of the buccal defect did not have any positive osteogenic effect. Apparently periosteal resection led to a situation where BMP-2 had a much lower osteogenic capacity, because periosteum contains high effective bone-forming progenitor and stem cells, which constitute a very useful source of bone regeneration in combination with BMP-2.[71]

Nonetheless, by using dogs in an animal experiment of this nature we encountered problems. Only 14 out of 24 implants could be retrieved for analysis, owing to overloading, i.e., owing to premature loss of the implants. The high level of implant loss was caused by early subclinical inflammation to the implant site, which could occur much more easily because of transgingival healing and uncontrolled immediate full loading. Although the dogs received soft diet and the implant site was controlled frequently, we noticed high levels of inflammation with loosening of the dental implants. This suggests that an animal model with transgingival healing of implants is not sufficient to investigate bone gain surrounding the implants. Because a transgingival healing modus was used, the implants were subjected to masticatory forces severely hampering osseointegration, which is described in a study conducted by Kim et al.[72] Compared with humans, the chewing patterns of dogs are also different, with increased transversal loading of implants even under a strict soft diet regime within the first 14 days after implantation. This should be kept in mind whenever dogs are used for animal studies with that kind of study protocol. As illustrated in Fig. 5, we inserted immediately loaded implants as well as unloaded implants on each side. But the latter also received instantaneous abutments. Even in the cases where implant abutments had no occlusal contact, unintentional loading by food or the tongue on the abutments was obvious. Especially in the initial phases after implant placement, such loading conditions can cause implant micromovement, resulting in subclinical infections and thereby disturbing the process of new bone formation. Nevertheless, the study design and the obtained data do not support the conclusion that BMP in general is ineffective in the regeneration process of alveolar bone defects.

CONCLUSION

The study design with rhBMP-2, released out of a CP carrier in a model system with transgingival inserted immediate and unloaded implants was ineffective, as it resulted in an incomplete regeneration of 5-6 mm buccal bone defects. Our data demonstrate that an application protocol of low dose rhBMP-2 even released out of a biomimetic organic carrier is not sufficient for enhancement of long-term stable bone formation at the bone implant interface in case of immediate loading. However, the study design and data do not support the conclusion that BMP in general will not work in alveolar bone defects.

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Title: Impact of rhBMP-2 on regeneration of buccal alveolar defects during the osseointegration of transgingival inserted implants
Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology

Dear Dr. Smeets,

I am writing concerning your paper, "Impact of rhBMP-2 on regeneration of buccal alveolar defects during the osseointegration of transgingival inserted implants" which you recently submitted to Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. Your paper has once again been carefully reviewed. I pleased to inform you that your revised paper has been accepted for publication the electronic version of the Journal.

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