The Effects of Recombinant Human Growth/Differentiation Factor-5 (RhGDF-5) on Bone Regeneration Around Titanium Dental Implants in Barrier Membrane–Protected Defects: A Pilot Study in the Mandible of Beagle Dogs

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Purpose: *This dog study sought to evaluate guided bone regeneration (GBR) in peri-implant defects following implantation of β-tricalcium phosphate (β-TCP) with and without osteoinductive recombinant human growth/differentiation factor-5 (rhGDF-5).* ***Materials and Methods:*** *In five beagle dogs, all mandibular premolars and the first molar were extracted. After 2 months, six buccolingual critical-size defects were created, and an implant was inserted into the center of each defect. One defect was filled with β-TCP coated with rhGDF-5 (600 μg/g β-TCP) and covered with a titanium-reinforced e-PTFE membrane (GDF group). A second defect received the same treatment, but pure uncoated β-TCP was used (TCP group). A third defect was filled with β-TCP mixed with autograft and not protected with a membrane (control group). The remaining three defects were filled with other biomaterials. After 2 months, total new bone area, regenerated bone height, and residual amount of β-TCP were determined histomorphometrically.* ***Results:*** *All implants osseointegrated. One membrane in each group became exposed. Mean new bone area for GDF, TCP, and control sites was 43.9 ± 18.7 mm^2, 32.3 ± 16.1 mm^2, and 13.1 ± 4.0 mm^2, respectively, with a significant difference between GDF and control groups. Mean regenerated bone height was 103.8 ± 29.7%, 75.4 ± 36.6%, and 67.2 ± 19.1% for the GDF, TCP, and control groups, respectively. Mean residual matrix volumes were 25.9 ± 13.6%, 30.0 ± 13.0%, and 13.4 ± 6.5%, respectively. Membrane protection of peri-implant defects filled with β-TCP resulted in a stronger effect on bone regeneration, although this was not statistically significant. The most pronounced regenerative results were achieved in rhGDF-5/β-TCP filled membrane-protected defects.* ***Conclusion:*** *Delivery of rhGDF-5 on β-TCP might have the potential to enhance the results of GBR in peri-implant defects.* Int J Oral Maxillofac Implants 2009;24:31–37

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Sufficient quality and quantity of bone are major prerequisites for the placement of dental implants into the alveolar ridge.[1] Bone volume can be increased by bone augmentation procedures either prior to or simultaneously with dental implant placement.[2,3] Among the techniques that have been employed to address this problem, the most recognized are autogenous bone grafting and guided bone regeneration (GBR).[4] In GBR, barrier membranes are used to prevent the ingrowth of competing soft tissue into bony defect areas.[5] Current clinical protocols often combine GBR with the use of autogenous bone. Osteoconductive bone substitutes have been evaluated as alternatives to autogenous bone.[6] These materials provide volume for new bone formation

and act as scaffolds for the ingrowth of osteoblasts. Regenerative results using solely osteoconductive bone substitutes are inferior to results achieved with autograft and are thus not satisfying.

A major focus of research has been the combination of osteoconductive scaffolds with osteoinductive proteins. These proteins can trigger the differentiation of mesenchymal stem cells and osteoprogenitor cells into osteoblasts and thus enhance the migration of dedicated bone-forming cells into the defect site.

Growth/differentiation factor-5 (GDF-5) is a member of the bone morphogenetic protein (BMP) superfamily, a group of proteins that is required for proper skeletal patterning and joint development in vertebrates.[7] Mutations in GDF-5 have been associated with skeletal abnormalities in both mice and humans.[8,9] Based on the activity of GDF-5 in skeletal development, a recombinant version of human GDF-5 (rhGDF-5) was evaluated for its osteoinductive potential both in vitro and in vivo.[10] In orthopedic animal models, the potential of rhGDF-5 to induce new bone formation after local implantation was demonstrated.[11–13] However, the osteoinductive properties of rhGDF-5 are also of interest for clinical application in dental and maxillofacial bone regeneration. To exert their effect in local bone regeneration, osteoinductive proteins have to be delivered using appropriate carrier systems. Beta tricalcium phosphate (β-TCP) is an ideal carrier for osteoinductive proteins, as it has excellent osteoconductive and space-providing properties. In addition, it is resorbable and can be replaced by viable new bone. Because β-TCP is a synthetic material, it poses no risk of pathogen transmission. β-TCP granules have been used in orthopedic and dental surgery for more than 20 years.[14] In an experimental study in mandibles of minipigs, β-TCP was the most promising material among the tested bone substitutes when used in combination with barrier membranes. An osteoinductive device combining rhGDF-5 with β-TCP has been successfully tested in sinus floor augmentation in the Goettingen minipig.[15]

The purpose of this study was to examine the bone regeneration potential of rhGDF-5 delivered on granular β-TCP in membrane-protected peri-implant bone defects in the canine mandible.

MATERIALS AND METHODS

Animals

Five female beagle dogs, 4 to 6 years in age and weighing approximately 11 to 15 kg, were used in this investigation. The study protocol was approved by the Ethical Committee of Biomatech (Chasse-sur-Rhone, France). (Biomatech was paid to provide animal housing and surgical facilities for the purpose of this study.) The study was performed in accordance with NF EN ISO 10993, part 2.[16]

Test Materials

β-tricalcium phosphate (β-TCP) and β-TCP coated homogeneously with rhGDF-5 were provided by Scil Technology (Martinsried, Germany). The β-TCP consisted of particles 500 to 1,000 μm in size with interconnecting porosity. The rhGDF-5 protein was coated onto the β-TCP in a concentration of 600 μg/g β-TCP using Scil Technology's proprietary technology.

Anesthetic Protocol

The dogs were sedated with atropine (0.05 mg/kg intramuscular; Aguettant, Lyon, France), and general anesthesia was induced with tiletamine-zolazepam (Zoletil 100, 5 to 10 mg/kg intramuscular; Virbac, Carros, France) followed by slow administration of sodium thiopental (Nesdonal 10 to 15 mg/kg intravenous; Merial, Lyon, France). Anesthesia was maintained by inhalation of a halothane (0.5 to 4%) mixture (Belamont, Boulogne Billancourt, France). To prevent hemorrhage, a local anesthetic agent and vasoconstrictor (lidocaine 2% with epinephrine 1:50,000) was injected into the surgical sites. Postsurgically, cephalexine (Rilexine 30 mg/kg; Virbac, Carros Cedex, France) was administered intravenously on the day of surgery and intramuscularly 5 days postsurgery. The anti-inflammatory agent flunixine (Finadyne 1 mg/kg intramuscular; Schering Plough, Levallois Perret, France) was administered for 3 days following surgery to reduce postsurgical pain.

Surgical Procedure

Two months prior to the beginning of the experiment, all four mandibular premolars (P1 to P4) and the first mandibular molar (M1) were extracted. The teeth were separated into a mesial and a distal part and the parts were carefully elevated out of the alveolar socket by means of elevators and forceps. The sockets were allowed to heal for 8 weeks. After 6 weeks, supragingival scaling was performed in all dogs.

On day zero (D0) of the study, a crestal full-thickness incision was made from the canine to the second molar under full anesthesia. Mucoperiosteal flaps were raised to the buccal and lingual sides. Three rectangular, saddle-type, through-and-through defects (5 mm apicocoronal, 8 mm mesiodistal, and 8 mm buccolingual at the bottom of the defects) were surgically created in the alveolar process of the edentulous area using a low-speed rotary drill and hand instruments. The defects were approximately 10 mm apart.

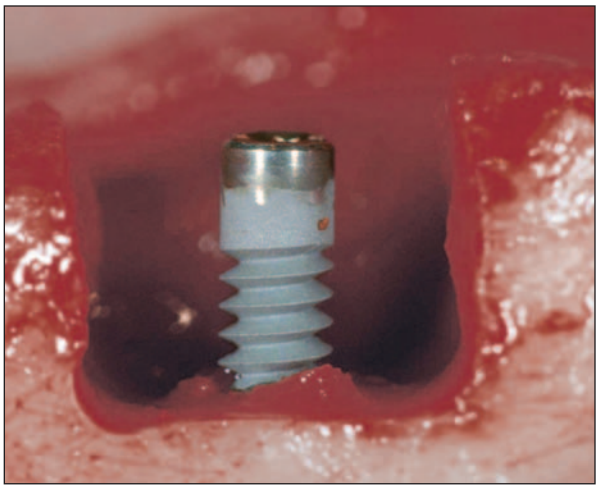

Fig 1 A 2 × 10-mm implant has been placed into the center of the defect. Note that half of the implant length is free-standing in the defect.

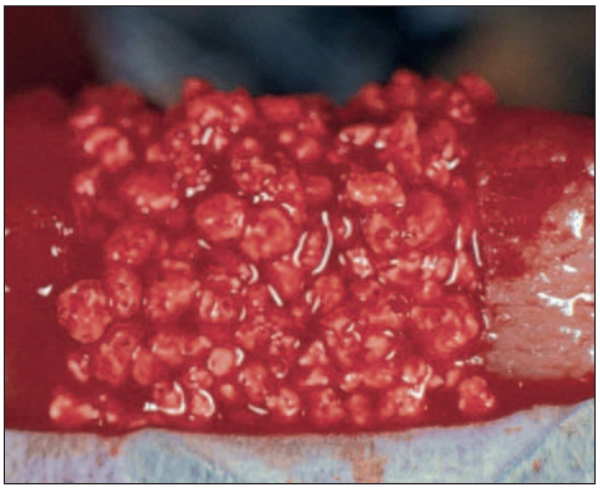

Fig 2 Defect in the GDF group, with β-TCP coated with rhGDF-5 filling the defect area surrounding the implant.

A custom-made endosseous oral implant (Osseotite, Implant Innovations, Palm Beach Gardens, FL) with a diameter of 2 mm and a length of 10 mm was inserted into each defect (Fig 1). The six defects were randomly assigned to various treatments. The first three were as follows:

1. β-TCP covered with a titanium-reinforced expanded polytetrafluoroethylene (e-PTFE) membrane (tr-GTAM, W. L. Gore & Associates, Flagstaff, AZ) (TCP group)
2. β-TCP coated with rhGDF-5 (600 μg/g β-TCP) and covered with a titanium-reinforced e-PTFE membrane (tr-GTAM, W. L. Gore & Associates) (GDF group) (Fig 2)
3. β-TCP mixed at a volume ratio of 1:1 with autogenous bone harvested from the prepared defects and ground up with a bone mill (control group)

The three remaining defects received other treatments and will be discussed in a similar study (unpublished data, 2003).

Approximately 500 mg of material was implanted into each defect. Each titanium-reinforced e-PTFE membrane was trimmed to the appropriate shape and draped over the ridge so that the membranes completely covered the defects and extended beyond the defect margins by approximately 3 mm. The membranes were stabilized with titanium minisscrews (Osseofix, Implant Innovations). Primary wound closure was achieved with vertical mattress and interrupted sutures (Gore-Tex sutures, W. L. Gore & Associates).

Postsurgical Procedures

The dogs were returned to their individual cages after implant placement. The dogs were observed clinically on a regular basis. Sutures were removed after 2 weeks. After 8 weeks, the dogs were weighed, anesthetized by intramuscular injection of Zoletil (50 mg/kg), and subsequently painlessly sacrificed by exsanguination. All dogs were subjected to macroscopic observation of thoracic, abdominal, and pelvic cavities and organs.

The heads of the animals were fixed by vascular perfusion with 10% formaldehyde in phosphate buffer pH 7 (Revol, Villeurbanne, France) through the carotid artery. The mandibles were then resected en bloc and subjected to histologic processing.

Histologic Processing and Histomorphometry

The block-resected mandibles were immersed in 10% neutral buffered formalin. After fixation, the specimens were washed in 0.185 mol/L sodium cacodylate buffer. The blocks were embedded in light-cured composite material (Technovit 7200 VLC; Kulzer, Wehrheim, Germany), and sections were cut, ground, and stained with toluidine blue (Sigma Chemical, Taufkirchen, Germany) according to a previously described method.[17] Buccolingual ground sections were made, and the two most central sections were evaluated histometrically and histomorphometrically.

Quantitative evaluation (histometric and histomorphometric) was performed with a light microscope (Metalloplan, Leitz, Wetzlar, Germany) equipped with a video camera (WV-CL500, Panasonic, Hamburg, Germany) and connected to a personal computer. The

Fig 3 Undecalcified sections from the control group (autogenous bone mixed with β-TCP). Bone formation was reduced and limited to triangular spaces next to the implant, owing to the lack of a membrane (toluidine blue; implant length 10 mm). *Arrow* = bottom of defect.

Fig 4 Undecalcified sections from the TCP group. Bone formation was limited. This applies to the new bone area but especially to the regenerated bone height (toluidine blue; implant length 10 mm). *Arrow* = bottom of defect.

transilluminated image from the light microscope was transferred in true color and real time over the video camera to a frame grabber board, where it was converted digitally. Image analysis was performed with the software ImageTool 3.0 (University of Texas Health Science Center, San Antonio, TX).

The following parameters were assessed:

- Bone area: Newly regenerated bone tissue buccal, lingual, and crestal to the implant, expressed in square millimeters. This area was delineated apically by the bottom of the defect.
- Bone height: Vertical height of newly regenerated bone tissue, measured from the bottom of the defect to the coronal extent of the bone tissue. Bone height is expressed in percentages, using the implant top as the 100% reference point.
- β-TCP: Area of residual β-TCP within the newly regenerated bone tissue, expressed as a percentage of bone area.

Statistical Analysis

Statistical evaluation was performed for the paired comparisons in the split-mouth design for the fixed factor treatment and the random factors side and position as part of an incomplete block design for more animals and more treatment groups using a mixed linear model. Two-sided all-pairs multiplicity-adjusted *P* values are reported for the treatment differences. All calculations were performed in R, a language and environment for statistical computing and graphics, using the library package lme4.

RESULTS

Clinical Observations

Clinical healing was uneventful, and no signs of nonosseointegrated implants were detected. In one animal of the GDF group and one in the TCP group, membrane exposure was visible at weeks 3 and 8, respectively, but the membranes were retained until sacrifice. In another animal of these groups, a fistula with no suppuration was present at sacrifice.

Histologic Observations

Control Group. A limited amount of new bone was visible with a marked reduction in bone width and height. Less residual carrier material was present than in the other groups as a result of the mixture with autogenous bone (Fig 3).

TCP Group. New bone formation in this group was reduced in both the total amount and the regenerated height. The ratio of the remaining carrier material within the newly formed bone tissue was similar to that seen in the GDF group. β-TCP particles not embedded in new bone tissue were almost completely resorbed. No inflammatory reactions were observed (Fig 4).

GDF Group. New bone formation was ample on the buccal and lingual aspects of the implant. The overall bone height exceeded the vertical implant height. The space provided by the membrane was completely filled with new trabecular bone tissue. Remaining carrier material was visible in the regenerated area. The newly formed bone showed character-

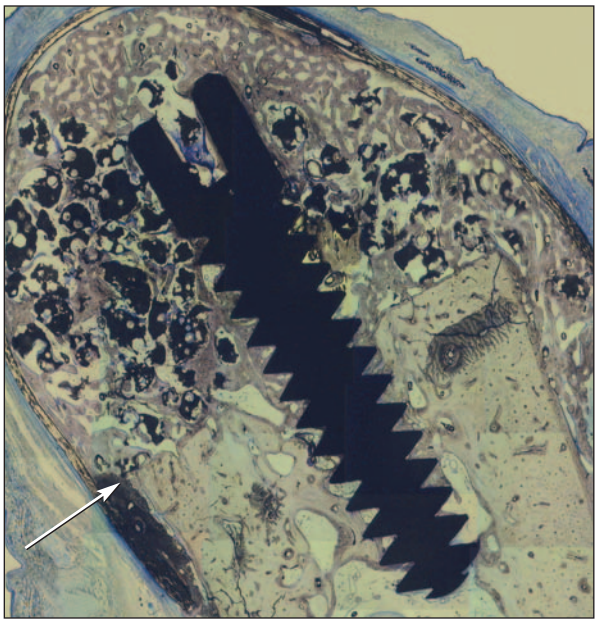

Fig 5 Undecalcified sections from a GDF group implant. Ample formation of new bone has occurred on all sides of this implant, with the new bone exceeding the implant top (toluidine blue; implant length 10 mm). *Arrow* = bottom of defect.

Table 1 Histomorphometric Analysis of Bone Area, Bone Height, and Residual β-TCP (Means ± SDs)

Measurement parameter	GDF group	TCP group	Control
Bone area (mm^2)	43.9 ± 18.7*	32.3 ± 16.1	13.1 ± 4.0*
Bone height (%)	103.8 ± 29.7	75.4 ± 36.6	67.2 ± 19.1
β-TCP (%)	25.9 ± 13.6	30.0 ± 13.0	13.4 ± 6.5

*Statistically significant difference between groups ($P \leq .05$).

istics of woven bone with occasionally embedded particles of the carrier material. No signs of inflammatory reactions were detected (Fig 5).

Histomorphometric Measurements

Means, standard deviations, and results of statistical analyses of the various histomorphometric measurements are provided in Table 1. Although no statistically significant difference between the GDF group and the TCP group was seen, a tendency toward more pronounced bone formation was obvious in the GDF group.

DISCUSSION

In this study the bone regeneration potential of the osteoinductive protein rhGDF-5 was evaluated in surgically created membrane-protected peri-implant defects in the canine mandible. The performance of rhGDF-5 delivered on β-TCP (GDF group) was compared to that of β-TCP in membrane-protected defects (TCP group). Unprotected defects filled with a mixture of β-TCP and autograft served as the control group. After 8 weeks of healing, more new bone had formed in the membrane-protected defects filled with rhGDF-5/β-TCP than in membrane-protected defects filled with β-TCP or in unprotected defects filled with β-TCP/autograft.

The surgical model used in this study is well accepted for the evaluation of bone grafts, either alone or in combination with membrane placement.[5,18] More recently, the effects of osteoinductive proteins and growth factors on bone regeneration have been assessed in comparable surgical models. The bone regeneration induced by rhTGF-β_1 delivered on a coral matrix carrier was evaluated by Ruskin et al.[19] Defects were either covered or not covered with a membrane. Significantly more bone regeneration was noted in membrane-protected defects as compared to defects without membrane coverage. However, it was not possible to detect any positive effect of rhTFG-β_1 on bone regeneration in membrane-protected defects. No notable difference in bone regeneration was observed between control and test sites. In contrast, in the present study a difference between the GDF and TCP groups was noted. New bone formation was increased in protected defects that had been filled with rhGDF-5/β-TCP as compared to protected defects filled with the carrier only. Jovanovic et al assessed the performance of recombinant human bone morphogenetic protein-2 (rhBMP-2) applied on a collagen sponge (ACS) in a full-thickness, saddle-type alveolar ridge defect model.[20] Histomorphometric analysis revealed average bone fill of 92% for GBR, whereas rhBMP-2/ACS alone or in combination with GBR resulted in 101% bone fill. Bone fill in surgical controls averaged 60%. Similar results were reported by Hunt et al for surgical controls.[21] Implantation of rhBMP-2, when applied on collagen or hyaluronic acid sponges, resulted in 100% and 94% bone fill, respectively. The analysis was based on two of three animals; the third animal was excluded since rhBMP-2 applied on either carrier

resulted in only limited bone fill of 62%. This animal was classified as an rhBMP-2 nonresponder of unknown nature.

The present study supported the finding that bone regeneration can be increased using bone substitutes, such as β-TCP, in combination with osteoinductive proteins and/or GBR. The new bone area in β-TCP–filled protected defects was notably greater than that seen in unprotected defects filled with β-TCP mixed with autograft. A further increase in new bone area was observed in the GDF group. Total regenerated bone height was comparable in the TCP and control groups. However, a marked increase in vertical bone height was achieved when the differentiation factor rhGDF-5 was added. This suggests that the addition of rhGDF-5 results in accelerated migration of bone-forming cells from resident bone into remote areas of the scaffold. The resulting early homogeneous bone regeneration might then contribute to the preservation of the initial graft volume.

A major shortcoming of GBR is the increased risk of infection subsequent to membrane exposure or wound dehiscence.[22] As previously described, in this study impaired regenerative results were observed in a site in the membrane-protected GDF and TCP groups, respectively, where early membrane exposure had occurred.[23] In addition, a fistula developed in one site of each membrane-protected group.

In the other three treatment groups, the bone-regeneration potential of rhGDF-5/β-TCP was evaluated in unprotected defects (unpublished data, 2003). It was demonstrated that limited vertical bone gain was achieved using β-TCP alone. The regenerative results obtained with rhGDF-5/β-TCP exceeded the results for β-TCP. Total new bone area approximated 25 mm^2 in rhGDF-5/β-TCP–filled defects and 18 mm^2 in β-TCP–filled defects. Total regenerated bone heights of 109% and 66% were achieved in defects filled with rhGDF-5/β-TCP and β-TCP, respectively.

CONCLUSIONS

Cochran et al assessed the effect of membrane protection on the regenerative potential of rhBMP-2 delivered on a collagen sponge on circular defects in the canine mandible.[24] A negative impact of the membrane at early time points was observed. A significantly reduced amount of new bone was noted in membrane-protected defects at 4 weeks, whereas at 12 weeks this reduction was less pronounced. It was suggested that the barrier membrane slows the bone regeneration process by delaying the immigration of BMP target cells, ie, precursor cells. It was clearly shown that the impact of rhBMP-2 on early bone formation was significantly higher in unprotected than in protected sites. This is in contrast with the findings of the present study and a similar study by Weng (unpublished data, 2003). If vertical bone gain was considered a parameter, the regenerative results achieved with rhGDF-5/β-TCP without membrane protection are comparable to the values achieved in the present study. On the other hand, a benefit of membrane protection was detected with respect to total new bone area. However, this benefit must be observed in conjunction with the obvious shortcomings of membrane-protected sites, ie, risk of infection and/or inflammation, which may compromise the regenerative results.

The bone substitute β-TCP was chosen as a carrier for rhGDF-5 because of several properties the material offers. The excellent biocompatibility of β-TCP was supported by the present findings. In addition, it was demonstrated in this study that, when β-TCP is combined with an osteoinductive protein, resorption of β-TCP is well balanced with bone ingrowth, thereby largely preserving vertical bone height. The amount of residual β-TCP was slightly decreased in the GDF group as compared to the TCP group. In the control group the residual β-TCP volume was approximately 50% of the value measured in the GDF group. This corresponds well with the initially lower β-TCP content of the filling material (50%).

It is concluded that the osteoinductive protein rhGDF-5 increases bone regeneration in membrane-protected alveolar ridge defects. As a result, rhGDF-5/β-TCP might have the potential to become a promising treatment modality in GBR of the alveolar ridge.

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