Efficacy of Growth Factor in Promoting Early Osseointegration

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A preclinical study was conducted to evaluate the feasibility of 2 different topical formulations of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) to promote early osseointegration and enhanced bone-to-implant contact (BIC) for dental implants placed in an edentulous ridge. Six female beagle dogs were divided into 3 groups. The control group included 4 implants with no coating; test group A included 10 implants with commercially available rhPDGF-BB formulation coating; and second test group B included 10 implants with prototype viscous rhPDGF-BB coating. Three dogs were sacrificed at 3 weeks (12 implants) and the remaining 3 dogs at 6 weeks after implant placement (12 implants). The specimens were retrieved for histological evaluation, and revealed an uneventful healing of all implants without any sign of an inflammatory response at the different time intervals. Furthermore, the bone was in very close contact with the implants' surfaces with no evidence of intervening fibrous tissue layers. At 3 weeks, new bone formation between most implant threads on rhPDGF-BB coated implants was evident, whereas in the control group only a thin and sparse amount of new bone was noted. At 6 weeks, the commercially available rhPDGF-BB formulation coated implant group (Group A) showed more trabecular bone and higher BIC compared to the other 2 groups. Histologically, the results in this study showed that use of conventionally available rhPDGF-BB formulation as the implant surface treatment may accelerate the process of osseointegration and enhance BIC.

Key Words: dental implant, bone regeneration, recombinant human platelet-derived growth factor-BB, histology, osseointegration

INTRODUCTION

Recent advances in tissue engineering have led to the utilization of growth factors to promote early osseointegration. Hall utilized the concept of coating dental implants with bioactive proteins with a titanium porous oxide oral implant surface as a carrier for recombinant human bone morphogenetic protein 2 (rhBMP-2) and rhBMP-7 to contribute an osteoinductive capacity to the implant surface.[1–3] There were other efforts to coat implants with growth factors with varied results.[4–6]

Recombinant human platelet-derived growth factor-BB (rhPDGF-BB) is widely researched, and it is considered the most potent of all isomeric forms.[7] This growth factor is commercially available (GEM 21S, Osteohealth, Shirley, NY), and the clinical efficacy and safety has been demonstrated for periodontal regeneration as well as in guided bone regeneration.[7–10]

There is no evidence in humans relative to the application of rhPDGF-BB as an implant surface coating. The aim of this preclinical canine study is to evaluate the feasibility of the topical application of

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DOI: 10.1563/AAID-JOI-D-13-00304

rhPDGF-BB to promote early osseointegration for dental implants placed in an edentulous ridge.

Materials and Methods

Study approval and outline

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the College of Dentistry, King Saud University, Riyadh, Saudi Arabia. Six female beagle dogs were divided into 3 groups. The control group (group C) included 4 implants with no coating, test group A included 10 implants with rhPDGF-BB coating (GEM21S, Osteohealth, Shirley, NY) and second test group B included 10 implants with prototype viscous rhPDGF-BB coating (Osteohealth).

Four premolars (P1-P4) and the first molar (M1) were atraumatically extracted under profound local and general anesthesia, and the areas were allowed to heal for a period of 6 weeks. A midcrestal incision was made on the healed alveolar ridge and a mucoperiosteal flap was reflected to expose the bone surface.

A total of 4 implants were inserted per animal (2 implants on each side) according to a randomized distribution pattern generated before surgery. The commercially available rhPDGF-BB formulation (Group A) and the prototype viscous rhPDGF-BB (Group B) were coated onto the implant surface and allowed to be absorbed for a minimum of 15 minutes before being delivered to the implant osteotomy sites. A total of 4 control implants (Group C) were placed without rhPDGF-BB coating. In addition, rhPDGF-BB was delivered to the osteotomy sites before implant insertion. The implants (tapered 3.4 mm in diameter $\times$ 8.5 mm in length, blasted, acid etched, and hydroxyapatite discrete crystal deposition, Biomet 3i, Palm Beach, FL) were placed at the level of the osseous crest mesially and distally using an insertion device and a hand ratchet according to the manufacturer guidelines. Resonance frequency analysis assessment was performed on all implants (Osstell AB, Göteborg, Sweden) before the healing abutments were placed. The healing abutments were connected (1-stage surgery) and the flaps were adapted for a tension-free wound closure with interrupted and horizontal mattress sutures (Vicryl Rapide, Ethicon, Somerville, NJ). The animals underwent a standard postsurgical infection and pain control protocol and the sutures were removed after 7–10 days. They were fed a soft diet throughout the study period, and were seen for postoperative visits at 2, 4, and 6 weeks after treatment. Three dogs were sacrificed at 3 weeks (12 implants) and the remaining 3 dogs at 6 weeks after implant placement (12 implants). The specimens were retrieved for histological evaluation.

Specimen preparation and analysis

The fixed samples were embedded following complete dehydration in ascending grades of ethanol (60%, 80%, 96%, and absolute ethanol) in a light-curing 1-component composite resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany). Polymerized blocks were initially ground to bring the tissue components closer to the cutting surface. A 100-μm-thick section attached to the second slide was cut with a diamond blade and 50 to 100 g of pressure. The final thickness of 40 um was achieved by grinding and final polishing with 1200-, 2400-, and 4000-grit sandpaper. Sections from each block were used for Sanderson's rapid bone stain (RBS) staining and acid fuchsin counterstain. Light microscopic overview images of the samples were taken digitally with a Leica M16 stereomicroscope (Leica Microsystems, Glattbrugg, Switzerland). The bone-to-implant contact (BIC) was calculated using the CT-Analyzer software (Leica SP 1600, Bannockburn, Ill). The analysis software assessed the total surface area of the region of interest (ROI) and the subset of the ROI surface that is intersected by binarized bone objects. The parameter thus measured was termed "intersection surface," which corresponded to the BIC. The BIC was calculated as the percentage of implant surface in contact with the bone through the whole perimeter of the implant at $\times$100 magnification.

Results

Clinical findings

All animals underwent an uneventful postoperative recovery. No adverse tissue reactions or clinical signs of inflammation were seen up until the time of sacrifice. All implants appeared to be stable and osseointegrated. Radiographs showed that all implant threads appeared to be maintained on the platform (Figures 1a and b). Resonance frequency

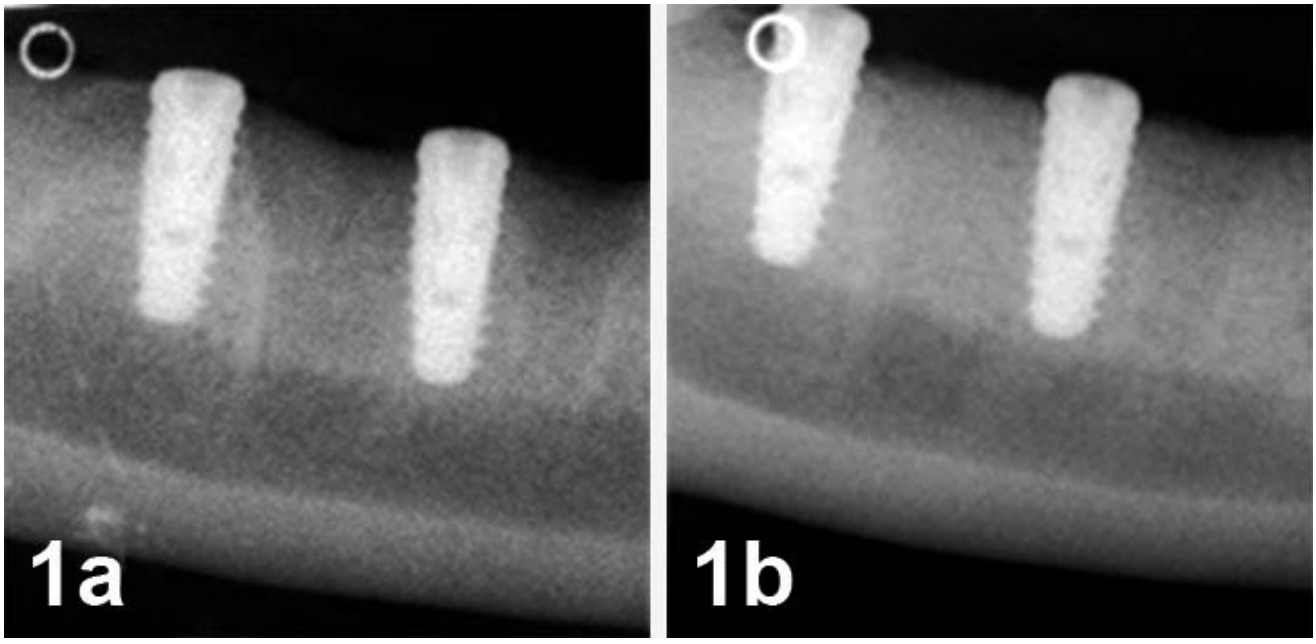

Figure 1. Periapical radiograph taken immediately after the surgery (a) and 6 weeks after the surgery for group A (b).

analysis assessment revealed ISQ values ranging from 70–84 immediately after the implant placement.

Histological evaluation

The histological evaluation revealed an uneventful healing of all implants without any signs of an inflammatory response at the different time intervals in all three groups (Figures 2a–3c). The bone was in very close contact with the implants surfaces with no evidence of intervening fibrous tissue layers. The newly-formed trabecular bone established bone–implant contact. Islands of native bone remained within the newly-formed bone and approached the implant surface at different distances. However, ground sections reveal differences in the qualitative surface topography between rhPDGF-BB coated and control implants. At 3 weeks, new bone formation between most implant threads on rhPDGF-BB coated implants was evident, whereas in the control group only a thin and sparse amount of new bone was noted (Figures 2a and b). The bone-to-implant contact (BIC) for group C was 58.7 $\pm$ 4.1%, 78.0 $\pm$ 12.5% for group A and 59.4 $\pm$ 17.6% for group B by microcomputed tomographic analysis. At 6 weeks, the commercially available rhPDGF-BB formulation coated implant group (Group A) showed more trabecular bone and higher BIC compared to other two groups (Figures 3a, b and c). The BIC was 59.9 $\pm$ 8.3% for group C, 69.8 $\pm$ 4.2% for group A and 62.0 $\pm$ 16.2% for group B.

Discussion

The purpose of the present study was to test the topical application of rhPDGF-BB to enhance early bone healing around dental implants. Two different formulations of rhPDGF-BBs were applied; FDA approved rhPDGF-BB formulation (group A) and prototype viscous rhPDGF-BB (group B). The results suggested the best osteogenic potential for the FDA approved rhPDGF-BB formulation coated implants (group A) at both time points (3 weeks and 6 weeks) compared to the implants without growth factor surface coating (group C) or prototype viscous rhPDGF-BB coated implants (group B). No obvious clinical and radiographic differences were detected between groups A and B, however, the BIC was higher in group A compared to group B when evaluated by micro computed tomographic analysis. Thus, the FDA approved rhPDGF-BB formulation appeared to induce higher BIC compared to the new prototype viscous rhPDGF-BB formulation. Although the handling aspect of the prototype viscous rhPDGF-BB formulation appeared to be enhanced, the histomorphometric result did not support its use for achieving better BIC.

Coating of dental implants with growth factors

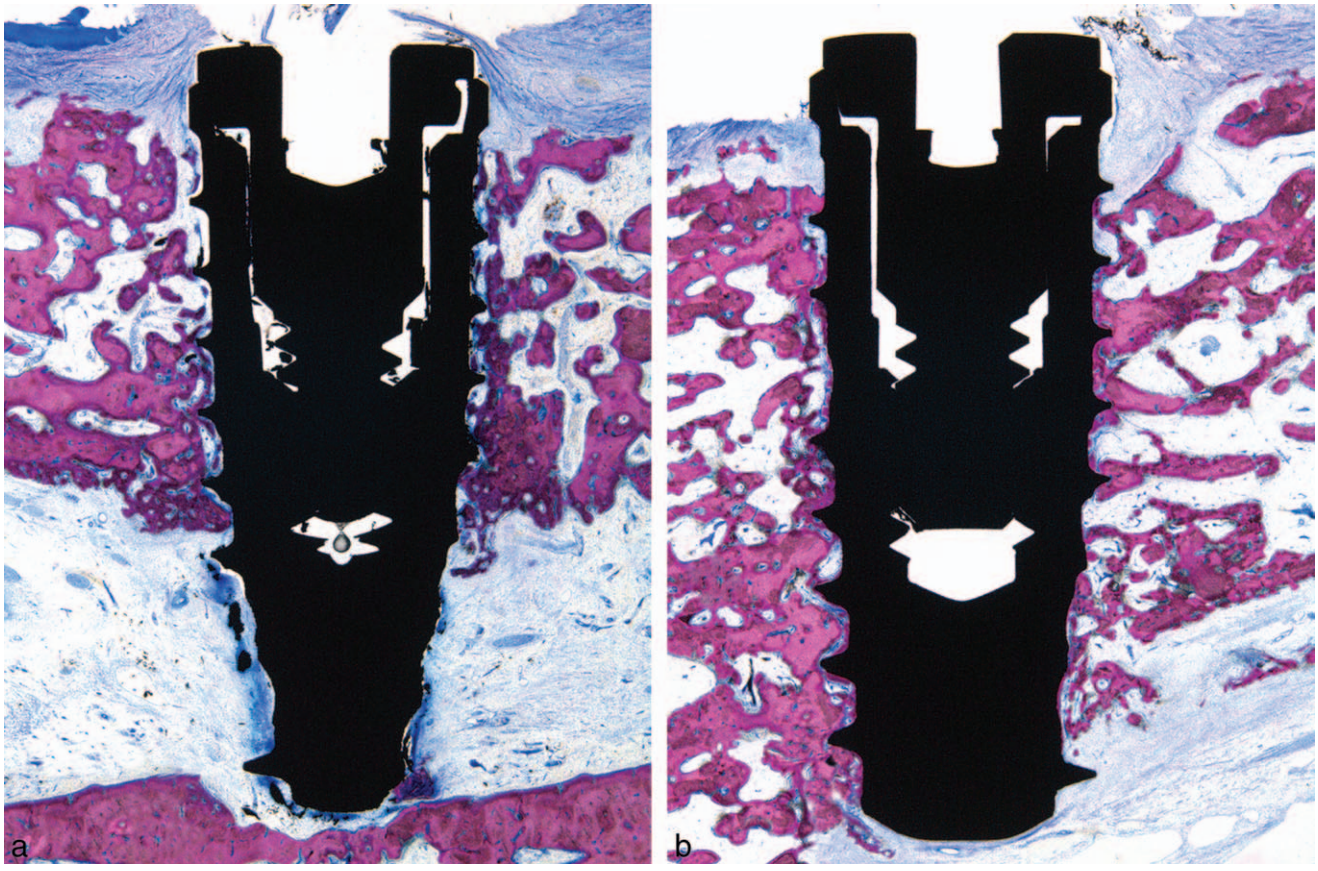

Figure 2. At 3 weeks post-implant placement, more bone-to-implant contact for the test group A (b) compared to the control group (a).

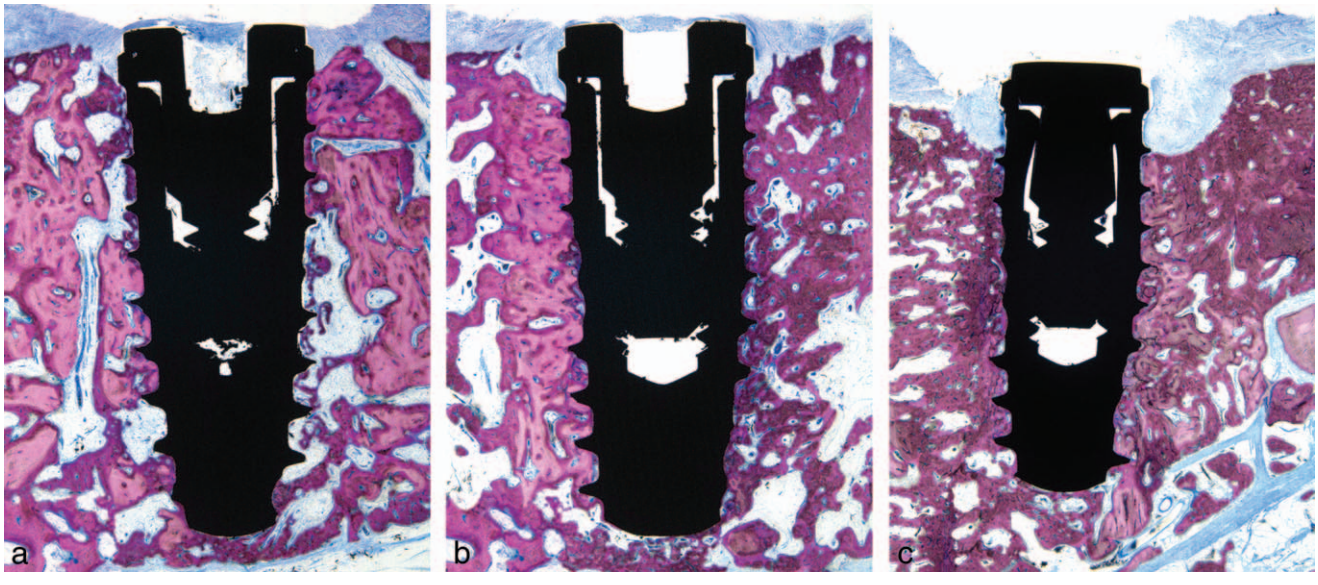

Figure 3. At 6 weeks post-implant placement, more trabecular bone and significant BIC can be seen in both test group A (b) and test group B (c) compared to the control group (a).

has revealed promising results in preclinical trials.[2–3] The objective of growth factor surface modifications is to enhance and improve the bone-implant contact, especially in the early postoperative period. Wikesjö demonstrated that rhBMP-2 coated implants have a higher osteoinductive capacity and the bone adjacent to implants exhibit improved bone density and BIC.[3] The extent of bone remodeling seemed to be correlated to the rhBMP-2 dose. This is in agreement with Hall *et al.,* 2007 showing that rhBMP-2 adsorbed onto titanium porous oxide surfaces led to a bone inductive effect including BIC contact which appeared to be surface- and dose dependent.[2]

In this study rhPDGF-BB was used as the implant surface coating. The authors are not able to verify the advantage of coating the implant osteotomy site prior to the implant insertion, and this concept needs to be addressed with follow-up studies. rhPDGF-BB provides chemotaxis, proliferation of osteogenic cells, and indirectly induces secretion of other growth factors by stimulating inflammatory cells such as macrophages in wound repair.[11–14] In addition, PDGF-BB is pro-angiogenic in that it acts in synergy with endogenous vascular epithelial growth factor (VEGF) to stimulate neovascularization at the defect site.[15–17] Marked bone re-modeling was observed at implants coated with two types of rhPDGF-BB. The histologic evaluation revealed evidence of accelerated bone re-modeling with islands of native bone in the immediate vicinity of the implant surface.

The present study used blasted, acid etched, and hydroxyapatite discrete crystal deposition surface implants as a vehicle for rhPDGF-BB. This implant surface may not only increase the contact area to bone but also incorporates biologic factors that enhance bone formation. Phipps et al[18] showed that adding hydroxyapatite to a polycaprolactone/collagen I (PCL/col) scaffold led to significantly more PDGF-BB adsorption, and subsequent release, with sustained release extending over an 8-week interval.

The design of this study did not allow for a meaningful statistical analysis to be performed for 3 different groups due to small sample size. However, the commercially available rhPDGF-BB formulation appeared to be effective in inducing early osseointegration when evaluated by histologic and micro CT analyses.

Conclusion

Results of this study showed that the implant surface that is utilized in this study can be a suitable carrier for rhPDGF-BB. This study also provides the first histologic evidence showing that use of rhPDGF-BB surface treatment improved initial bone formation and enhanced early osseointegration.

Abbreviations

BIC: bone-to-implant contact
rhBMP: recombinant human bone morphogenic protein
rhPDGF-BB: recombinant human platelet derived growth factor-BB
ROI: region of interest

Acknowledgments

This study was sponsored by grants from Osteohealth and College of Dentistry Research Center, King Saud University. Authors would also like to thank Biomet 3i for donating dental implants. Authors reported no conflict of interest.

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